

HISTONE DEACETYLASE INHIBITORS

RELATED APPLICATION

[001] This application claims the benefit of U.S. Provisional Application No. 60/419,929, filed October 21, 2002, which is incorporated herein by reference.

FIELD OF THE INVENTION

[002] The invention relates to compounds that may be used to inhibit histone deacetylases (HDAC) as well as compositions of matter and kits comprising these compounds. The present invention also relates to methods for inhibiting HDAC as well as treatment methods using compounds according to the present invention.

DESCRIPTION OF RELATED ART

[003] DNA in eukaryotic cells is tightly complexed with proteins (histones) to form chromatin. Histones are small, positively charged proteins that are rich in basic amino acids (positively charged at physiological pH), which contact the phosphate groups (negatively charged at physiological pH) of DNA. There are five main classes of histones H1, H2A, H2B, H3, and H4. The amino acid sequences of H2A, H2B, H3, and H4 show remarkable conservation between species, wherein H1 varies somewhat and in some cases is replaced by another histone, e.g., H5. Four pairs of each of H2A, H2B, H3 and H4 together form a disk-shaped octomeric protein core, around which DNA (about 140 base pairs) is wound to form a nucleosome. Individual nucleosomes are connected by short stretches of linker DNA associated with another histone molecule to form a structure resembling a beaded string, which is itself arranged in a helical stack, known as a solenoid.

[004] The majority of histones are synthesized during the S phase of the cell cycle, and newly synthesized histones quickly enter the nucleus to become associated with DNA. Within minutes of its synthesis, new DNA becomes associated with histones in nucleosomal structures.

[005] A small fraction of histones, more specifically, the amino acid side chains thereof, are enzymatically modified by post-translational addition of methyl, acetyl, or phosphate groups, neutralizing the positive charge of the side chain, or converting it to a negative charge. For example, lysine and arginine groups may be methylated, lysine groups may be acetylated, and

serine groups may be phosphorylated. For lysine, the -(CH₂)₄-NH₂ sidechain may be acetylated, for example by an acetyltransferase enzyme to give the amide -(CH₂)₄-NHC(=O)CH₃.

Methylation, acetylation, and phosphorylation of amino termini of histones that extend from the nucleosomal core affects chromatin structure and gene expression. Spencer and Davie 1999.

Gene 240:1 1-12.

[006] Acetylation and deacetylation of histones is associated with transcriptional events leading to cell proliferation and/or differentiation. Regulation of the function of transcriptional factors is also mediated through acetylation. Recent reviews on histone deacetylation include Kouzarides, et al., 1999. Curr. Opin. Genet. Dev. 9:1, 40-48 and Pazin, et al. 1997. 89:3 325-328.

[007] The correlation between acetylation status of histones and the transcription of genes has been known for quite some time. Certain enzymes, specifically acetylases (e.g., histone acetyltransferases (HAT) and deacetylases (histone deacetylase or HDAC), which regulate the acetylation state of histones have been identified in many organisms and have been implicated in the regulation of numerous genes, confirming a link between acetylation and transcription. In general, histone acetylation is believed to correlate with transcriptional activation, whereas histone deacetylation is believed to be associated with gene repression.

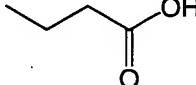
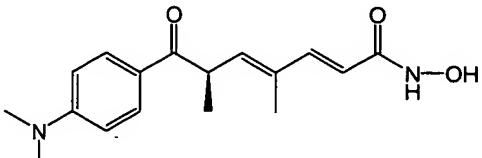
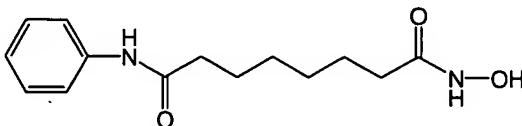
[008] A growing number of histone deacetylases (HDACs) have been identified. HDACs function as part of large multiprotein complexes, which are tethered to the promoter and repress transcription. Well characterized transcriptional repressors such as MAD, nuclear receptors and YY1 associate with HDAC complexes to exert their repressor function.

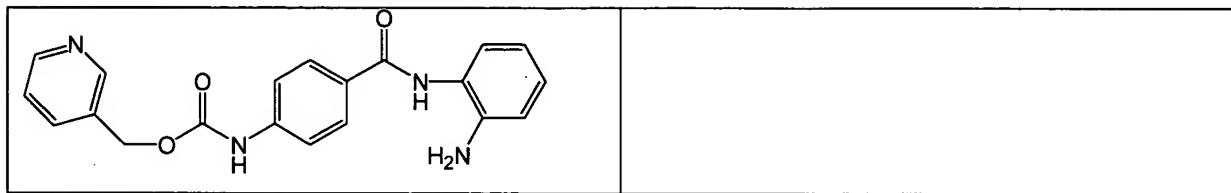
[009] Studies of HDAC inhibitors have shown that these enzymes play an important role in cell proliferation and differentiation. HDACs are believed to be associated with a variety of different disease states including, but not limited to cell proliferative diseases and conditions (Marks, P.A., Richon, V.M., Breslow, R. and Rifkind, R.A., J. Natl. Cancer Inst. (Bethesda) 92, 1210-1215, 2000) such as leukemia (Lin et al. 1998. Nature 391: 811-814; Grignani, et al. 1998. Nature 391: 815-818; Warrell et al. 1998. J. Natl. Cancer Inst. 90:1621-1625; Gelmetti et al. 1998. Mol. Cell Biol. 18:7185-7191; Wang et al. 1998. PNAS 951 0860-10865), melanomas/squamous cell carcinomas (Gillenwater, et al., 1998, Int. J. Cancer 75217-224; Saunders, et al., 1999, Cancer Res. 59:399-404), breast cancer, prostate cancer, bladder cancer (Gelmetti et al. 1998. Mol. Cell Biol. 18:7185-7191; Wang et al. 1998. PNAS 951 0860-10865),

lung cancer, ovarian cancer and colon cancer (Hassig, et al., 1997, Chem. Biol. 4:783-789; Archer, et al., 1998, PNAS, 95:6791-6796; Swendeman, et al., 1999, Proc. Amer. Assoc. Cancer Res. 40, Abstract #3836).

[0010] Histone deacetylase inhibitors are potent inducers of growth arrest, differentiation, or apoptotic cell death in a variety of transformed cells in culture and in tumor bearing animals (*Histone deacetylase inhibitors as new cancer drugs*, Marks, P.A., Richon, V.M., Breslow, R. and Rifkind, R.A., Current Opinions in Oncology, 2001, Nov. 13 (6): 477-83; *Histone deacetylases and cancer: causes and therapies*, Marks, P., Rifkind, R.A., Richon, V.M., Breslow, R., Miller, T. and Kelly, W.K., Nat. Rev. Cancer 2001 Dec. 1 (3):194-202). In addition, HDAC inhibitors are useful in the treatment or prevention of protozoal diseases (US Patent 5,922,837) and psoriasis (PCT Publication No. WO 02/26696).

[0011] A variety of inhibitors of HDAC have been reported. Some of these inhibitors are described in the following table:

Inhibitors	References
Butyrates 	Marks PA, et al., J. Natl. Cancer Inst. 2000, 92:1210–1216; Weidle UH, et al., Anticancer Res. 2000, 20:1471–1486; Gore SD, et al., Exp. Opin. Invest. Drugs 2000, 9:2923–2934; Sowa Y, et al., Biofactors 2000, 12:283–287
Trichostatin A 	Marks PA, et al., J. Natl. Cancer Inst. 2000, 92:1210–1216; Weidle UH, et al., Anticancer Res. 2000, 20:1471–1486; Nervi C, et al., Cancer Res. 2001, 61:1247–1249; Suzuki T, et al., Int. J. Cancer 2000, 88:992–997.
Suberoylanilidine hydroxamic acid 	Marks PA, et al., J. Natl. Cancer Inst. 2000, 92:1210–1216; Kelly WK, et al., Proc. Amer. Soc. Clin. Oncol. 2001, 20:87a; Butler LM, et al., Cancer Res. 2000, 60:5165–5170.
MS-275	Lee BI, et al., Cancer Res. 2001, 61:931–934.



[0012] Additional examples of HDAC inhibitors can be found in Marks PA, et al., J. Natl. Cancer Inst. 2000, 92:1210–1216 & Weidle UH, et al., Anticancer Res. 2000, 20:1471–1486 and PCT Publication Nos. WO 02/26696, WO 02/062773, and WO 01/18171.

[0013] Despite the various HDAC inhibitors that have been reported to date, a need continues to exist for new and more effective inhibitors of HDACs.

SUMMARY OF THE INVENTION

[0014] The present invention relates to compounds that have activity for inhibiting HDACs. The present invention also provides compositions, articles of manufacture and kits comprising these compounds.

[0015] In one embodiment, a pharmaceutical composition is provided that comprises a HDAC inhibitor according to the present invention as an active ingredient. Pharmaceutical compositions according to the invention may optionally comprise 0.001%-100% of one or more HDAC inhibitors of this invention. These pharmaceutical compositions may be administered or coadministered by a wide variety of routes, including for example, orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local delivery (for example by catheter or stent), subcutaneously, intraadiposally, intraarticularly, or intrathecally. The compositions may also be administered or coadministered in slow release dosage forms.

[0016] The invention is also directed to kits and other articles of manufacture for treating disease states associated with HDAC.

[0017] In one embodiment, a kit is provided that comprises a composition comprising at least one HDAC inhibitor of the present invention in combination with instructions. The instructions may indicate the disease state for which the composition is to be administered, storage information, dosing information and/or instructions regarding how to administer the

composition. The kit may also comprise packaging materials. The packaging material may comprise a container for housing the composition. The kit may also optionally comprise additional components, such as syringes for administration of the composition. The kit may comprise the composition in single or multiple dose forms.

[0018] In another embodiment, an article of manufacture is provided that comprises a composition comprising at least one HDAC inhibitor of the present invention in combination with packaging materials. The packaging material may comprise a container for housing the composition. The container may optionally comprise a label indicating the disease state for which the composition is to be administered, storage information, dosing information and/or instructions regarding how to administer the composition. The kit may also optionally comprise additional components, such as syringes for administration of the composition. The kit may comprise the composition in single or multiple dose forms.

[0019] Also provided are methods for preparing compounds, compositions and kits according to the present invention. For example, several synthetic schemes are provided herein for synthesizing compounds according to the present invention.

[0020] Also provided are methods for using compounds, compositions, kits and articles of manufacture according to the present invention.

[0021] In one embodiment, the compounds, compositions, kits and articles of manufacture are used to inhibit HDAC.

[0022] In one embodiment, the compounds, compositions, kits and articles of manufacture are used to treat a disease state for which HDAC possesses activity that contributes to the pathology and/or symptomology of the disease state.

[0023] In another embodiment, a compound is administered to a subject wherein HDAC activity within the subject is altered, preferably reduced.

[0024] In another embodiment, a prodrug of a compound is administered to a subject that is converted to the compound *in vivo* where it inhibits HDAC.

[0025] In another embodiment, a method of inhibiting HDAC is provided that comprises contacting HDAC with a compound according to the present invention.

[0026] In another embodiment, a method of inhibiting HDAC is provided that comprises causing a compound according to the present invention to be present in a subject in order to inhibit HDAC *in vivo*.

[0027] In another embodiment, a method of inhibiting HDAC is provided that comprises administering a first compound to a subject that is converted *in vivo* to a second compound wherein the second compound inhibits HDAC *in vivo*.

[0028] In another embodiment, a therapeutic method is provided that comprises administering a compound according to the present invention.

[0029] In another embodiment, a method of inhibiting cell proliferation is provided that comprises contacting a cell with an effective amount of a compound according to the present invention.

[0030] In another embodiment, a method of inhibiting cell proliferation in a patient is provided that comprises administering to the patient a therapeutically -effective amount of a compound according to the present invention.

[0031] In another embodiment, a method of treating a condition in a patient which is known to be mediated by HDAC, or which is known to be treated by HDAC inhibitors, comprising administering to the patient a therapeutically -effective amount of a compound according to the present invention.

[0032] In another embodiment, a method is provided for using a compound according to the present invention in order to manufacture a medicament for use in the treatment of disease state which is known to be mediated by HDAC, or which is known to be treated by HDAC inhibitors.

[0033] In another embodiment, a method is provided for treating a disease state for which HDAC possesses activity that contributes to the pathology and/or symptomology of the disease state, the method comprising: causing a compound according to the present invention to be present in a subject in a therapeutically effective amount for the disease state.

[0034] In another embodiment, a method is provided for treating a disease state for which HDAC possesses activity that contributes to the pathology and/or symptomology of the disease state, the method comprising: administering a first compound to a subject that is converted *in*

vivo to a second compound such that the second compound is present in the subject in a therapeutically effective amount for the disease state.

[0035] In another embodiment, a method is provided for treating a disease state for which HDAC possesses activity that contributes to the pathology and/or symptomology of the disease state, the method comprising: administering a compound according to the present invention to a subject such that the compound is present in the subject in a therapeutically effective amount for the disease state.

[0036] In another embodiment, a method is provided for treating a cell proliferative disease state comprising treating cells with a compound according to the present invention in combination with an anti-proliferative agent, wherein the cells are treated with the compound according to the present invention before, at the same time, and/or after the cells are treated with the anti-proliferative agent, referred to herein as combination therapy. It is noted that treatment of one agent before another is referred to herein as sequential therapy, even if the agents are also administered together. It is noted that combination therapy is intended to cover when agents are administered before or after each other (sequential therapy) as well as when the agents are administered at the same time.

[0037] Examples of diseases that may be treated by administration of compounds and compositions according to the present invention include, but are not limited to protozoal diseases and cell proliferative diseases and conditions such as leukemia, melanomas, squamous cell carcinomas, breast cancer, prostate cancer, bladder cancer, lung cancer, ovarian cancer and colon cancer.

[0038] It is noted in regard to all of the above embodiments that the present invention is intended to encompass pharmaceutically acceptable salts and solvates (e.g., hydrates) of the compounds, regardless of whether such salts and solvates are specified since it is well known in the art to administer pharmaceutical agents in a salt or solvated form. It is further noted that prodrugs may also be administered and become a compound according to the present invention. The various methods of using the compounds of the present invention are intended, regardless of whether prodrug delivery is specified, to encompass the administration of a prodrug that is converted *in vivo* into a compound according to the present invention.

BRIEF DESCRIPTION OF THE FIGURES

- [0039] Figure 1 illustrates a ribbon diagram overview of the structure of HDAC8, highlighting the secondary structural elements of the protein.
- [0040] Figure 2A illustrates particular examples of substituents R₁, R₂, R₃, R₄ and R₅ that may be employed.
- [0041] Figure 2B illustrates examples of moieties, J, that the linker group may comprise to link the linker group to the remainder of the compound.
- [0042] Figure 2C illustrates particular examples of moieties that the leader groups may comprise.
- [0043] It is noted in regard to Figures 2A-2C that the squiggle line is intended to indicate a bond to an adjacent moiety. It is also noted that the substituents shown may optionally be further substituted beyond what is shown. Further, one or more heteroatoms may optionally be substituted for the carbon atoms shown. In regard to Figure 2C, it is noted that the leader groups moieties may be incorporated into the leader group in either possible orientation.
- [0044] Figure 2D illustrates particular examples of compounds according to the present invention.
- [0045] Figure 3 illustrates the DNA and amino acid sequence of HDAC8.
- [0046] Figure 4 illustrates the protein sequence shown in SEQ. ID No. 3.
- [0047] Figure 5 provides examples of compounds that were synthesized and found to have better than 200 nM HDAC8 activity according to the assay described in example 2.

DEFINITIONS

- [0048] Unless otherwise stated, the following terms used in the specification and claims shall have the following meanings for the purposes of this Application.
- [0049] "Alicyclic" means a moiety comprising a non-aromatic ring structure. Alicyclic moieties may be saturated or partially unsaturated with one, two or more double or triple bonds. Alicyclic moieties may also optionally comprise heteroatoms such as nitrogen, oxygen and sulphur. Examples of alicyclic moieties include, but are not limited to moieties with C3 - C8 rings such as cyclopropyl, cyclohexane, cyclopentane, cyclopentene, cyclopentadiene,

cyclohexane, cyclohexene, cyclohexadiene, cycloheptane, cycloheptene, cycloheptadiene, cyclooctane, cyclooctene, and cyclooctadiene.

[0050] "Alkyl" represented by itself means a straight or branched, saturated or unsaturated, aliphatic radical having a chain of carbon atoms, optionally with oxygen (See "oxaalkyl") or nitrogen atoms (See "aminoalkyl") between the carbon atoms. C_x alkyl and C_{x-y} alkyl are typically used where X and Y indicate the number of carbon atoms in the chain. For example, C_{1-6} alkyl includes alkyls that have a chain of between 1 and 6 carbon (e.g., methyl, ethyl, propyl, isopropyl, butyl, *sec*-butyl, isobutyl, *tert*-butyl, vinyl, allyl, 1-propenyl, isopropenyl, 1-butenyl, 2-butenyl, 3-butenyl, 2-methylallyl, ethynyl, 1-propynyl, 2-propynyl, and the like). Alkyl represented along with another radical (e.g., as in arylalkyl) means a straight or branched, saturated or unsaturated aliphatic divalent radical having the number of atoms indicated or when no atoms are indicated means a bond (e.g., $(C_{6-10})\text{aryl}(C_{0-3})\text{alkyl}$ includes phenyl, benzyl, phenethyl, 1-phenylethyl 3-phenylpropyl, and the like).

[0051] "Alkylene", unless indicated otherwise, means a straight or branched, saturated or unsaturated, aliphatic, divalent radical. C_x alkylene and C_{x-y} alkylene are typically used where X and Y indicate the number of carbon atoms in the chain. For example, C_{1-6} alkylene includes methylene (-CH₂-), ethylene (-CH₂CH₂-), trimethylene (-CH₂CH₂CH₂-), tetramethylene (-CH₂CH₂CH₂CH₂-) 2-butenylene (-CH₂CH=CHCH₂-), 2-methyltetramethylene (-CH₂CH(CH₃)CH₂CH₂-), pentamethylene (-CH₂CH₂CH₂CH₂CH₂-) and the like).

[0052] "Alkylidene" means a straight or branched saturated or unsaturated, aliphatic, divalent radical. C_x alkylidene and C_{x-y} alkylidene are typically used where X and Y indicate the number of carbon atoms in the chain. For example, C_{1-6} alkylidene includes methylene (=CH₂), ethyldene (=CHCH₃), isopropylidene (=C(CH₃)₂), propylidene (=CHCH₂CH₃), allylidene (=CHCH=CH₂), and the like).

[0053] "Amino" means a nitrogen moiety having two further substituents where a hydrogen or carbon atom is alpha to the nitrogen. Unless indicated otherwise, the compounds of the invention containing amino moieties may include protected derivatives thereof. Suitable protecting groups for amino moieties include acetyl, *tert*-butoxycarbonyl, benzyloxycarbonyl, and the like.

[0054] "Aminoalkyl" means an alkyl, as defined above, except where one or more substituted or unsubstituted nitrogen atoms (-N-) are positioned between carbon atoms of the alkyl. For example, an (C₂₋₆) aminoalkyl refers to a chain comprising between 2 and 6 carbons and one or more nitrogen atoms positioned between the carbon atoms.

[0055] "Animal" includes humans, non-human mammals (e.g., dogs, cats, rabbits, cattle, horses, sheep, goats, swine, deer, and the like) and non-mammals (e.g., birds, and the like).

[0056] "Aromatic" means a moiety wherein the constituent atoms make up an unsaturated ring system, all atoms in the ring system are *sp*² hybridized and the total number of pi electrons is equal to 4n+2.

[0057] "Aryl" means a monocyclic or fused bicyclic ring assembly wherein each ring is comprised of 5 or 6 membered ring and is aromatic or when fused with a second ring forms an aromatic ring assembly. When the aryl comprises heteroatoms (e.g., N, O or S), the aryl is a heteroaryl. C_X aryl and C_{X-Y} aryl are typically used where X and Y indicate the number of carbon atoms in ring and directly attached to the ring. For example, optionally substituted (C₆₋₁₀)aryl as used in this Application includes, but is not limited to, biphenyl-2-yl, 2-bromophenyl, 2-bromocarbonylphenyl, 2-bromo-5-fluorophenyl, 4-*tert*-butylphenyl, 4-carbamoylphenyl, 4-carboxy-2-nitrophenyl, 2-chlorophenyl, 4-chlorophenyl, 3-chlorocarbonylphenyl, 4-chlorocarbonylphenyl, 2-chloro-4-fluorophenyl, 2-chloro-6-fluorophenyl, 4-chloro-2-nitrophenyl, 6-chloro-2-nitrophenyl, 2,6-dibromophenyl, 2,3-dichlorophenyl, 2,5-dichlorophenyl, 3,4-dichlorophenyl, 2-difluoromethoxyphenyl, 3,5-dimethylphenyl, 2-ethoxycarbonylphenyl, 2-fluorophenyl, 2-iodophenyl, 4-isopropylphenyl, 2-methoxyphenyl, 4-methoxyphenyl, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 5-methyl-2-nitrophenyl, 4-methylsulfonylphenyl, naphth-2-yl, 2-nitrophenyl, 3-nitrophenyl, 4-nitrophenyl, 2,3,4,5,6-pentafluorophenyl, phenyl, 2-trifluoromethoxyphenyl, 3-trifluoromethoxyphenyl, 4-trifluoromethoxyphenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 2-trifluoromethylsulfanylphenyl, 4-trifluoromethylsulfanylphenyl, and the like.

[0058] "Bicycloaryl" means a bicyclic ring assembly wherein the rings are linked by a single bond or fused and at least one of the rings comprising the assembly is aromatic. C_X bicycloaryl and C_{X-Y} bicycloaryl are typically used where X and Y indicate the number of carbon

atoms in the bicyclic ring assembly and directly attached to the ring. For example, C₉₋₁₀ bicycloaryl includes cyclohexylphenyl, 1,2-dihydroronaphthyl, 2,4-dioxo-1,2,3,4-tetrahydronaphthyl, indanyl, indenyl, 1,2,3,4-tetrahydronaphthyl, and the like.

[0059] "Cycloalkyl" means a saturated or partially unsaturated, monocyclic, fused bicyclic or bridged polycyclic ring assembly. C_X cycloalkyl and C_{X-Y} cycloalkyl are typically used where X and Y indicate the number of carbon atoms in the ring assembly. For example, C₃₋₁₀ cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, 2,5-cyclohexadienyl, bicyclo[2.2.2]octyl, adamantan-1-yl, decahydronaphthyl, oxocyclohexyl, dioxocyclohexyl, thiocyclohexyl, 2-oxobicyclo[2.2.1]hept-1-yl, and the like.

[0060] "Cycloalkylene" means a divalent saturated or partially unsaturated, monocyclic ring or bridged polycyclic ring assembly. C_X cycloalkylene and C_{X-Y} cycloalkylene are typically used where X and Y indicate the number of carbon atoms in the ring assembly.

[0061] "Disease" specifically includes any unhealthy condition of an animal or part thereof and includes an unhealthy condition that may be caused by, or incident to, medical or veterinary therapy applied to that animal, i.e., the "side effects" of such therapy.

[0062] "Halo" or "Halogen" means fluoro, chloro, bromo or iodo.

[0063] "Halo-substituted alkyl", as an isolated group or part of a larger group, means "alkyl" substituted by one or more "halo" atoms, as such terms are defined in this Application. Halo-substituted alkyl includes haloalkyl, dihaloalkyl, trihaloalkyl, perhaloalkyl and the like (e.g. halo-substituted (C₁₋₃)alkyl includes chloromethyl, dichloromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, perfluoroethyl, 2,2,2-trifluoro-1,1-dichloroethyl, and the like).

[0064] "Heteroatom" refers to an atom that is not a carbon atom. Particular examples of heteroatoms include, but are not limited to nitrogen, oxygen, and sulphur.

[0065] "Heteroatom moiety" includes a moiety where the atom by which the moiety is attached is not a carbon. Examples of heteroatom moieties include -N=, -NR-, -N⁺(O)=, -O-, -S- or -S(O)₂-, wherein R is further substituent.

[0066] "Heterocycloalkylene" means cycloalkylene, as defined in this Application, provided that one or more of the ring member carbon atoms indicated, is replaced by a heteroatom.

[0067] "Heteroaryl" means a five or six membered aromatic ring, as defined in this Application, provided that one or more of the ring carbon atoms indicated are replaced by a heteroatom. For example, optionally substituted hetero(C₅₋₁₃)aryl as used in this Application includes, but is not limited to, 4-amino-2-hydroxypyrimidin-5-yl, dibenzofuranyl, benzothiazol-2-yl, 1*H*-benzoimidazol-2-yl, 2-bromopyrid-5-yl, 5-bromopyrid-2-yl, 4-carbamoylthiazol-2-yl, 3-carboxypyrid-4-yl, 5-carboxy-2,6-dimethylpyrid-3-yl, 3,5-dimethylisoxazol-4-yl, 5-ethoxy-2,6-dimethylpyrid-3-yl, 5-fluoro-6-hydroxypyrimidin-4-yl, fur-2-yl, fur-3-yl, 5-hydroxy-4,6-dimethylpyrid-3-yl, 8-hydroxy-5,7-dimethylquinolin-2-yl, 5-hydroxymethylisoxazol-3-yl, 3-hydroxy-6-methylpyrid-2-yl, 3-hydroxypyrid-2-yl, 1*H*-imidazol-2-yl, 1*H*-imidazol-4-yl, 1*H*-indol-3-yl, isothiazol-4-yl, isoxazol-4-yl, 2-methylfur-3-yl, 5-methylfur-2-yl, 1-methyl-1*H*-imidazol-2-yl, 5-methyl-3*H*-imidazol-4-yl, 5-methylisoxazol-3-yl, 5-methyl-2*H*-pyrazol-3-yl, 3-methylpyrid-2-yl, 4-methylpyrid-2-yl, 5-methylpyrid-2-yl, 6-methylpyrid-2-yl, 2-methylpyrid-3-yl, 2-methylthiazol-4-yl, 5-nitropyrid-2-yl, 2*H*-pyrazol-3-yl, 3*H*-pyrazol-4-yl, pyridazin-3-yl, pyrid-2-yl, pyrid-3-yl, pyrid-4-yl, 5-pyrid-3-yl-2*H*-[1,2,4]triazol-3-yl, pyrimidin-4-yl, pyrimidin-5-yl, 1*H*-pyrrol-3-yl, quinolin-2-yl, 1*H*-tetrazol-5-yl, thiazol-2-yl, thiazol-5-yl, thien-2-yl, thien-3-yl, 2*H*-[1,2,4]triazol-3-yl, 3*H*-[1,2,3]triazol-4-yl, 5-trifluoromethylpyrid-2-yl, and the like. Suitable protecting groups include *tert*-butoxycarbonyl, benzyloxycarbonyl, benzyl, 4-methoxybenzyl, 2-nitrobenzyl, and the like.

[0068] "Heterobicycloaryl" means bicycloaryl, as defined in this Application, provided that one or more of the ring carbon atoms indicated are replaced by a heteroatom. For example, hetero(C₈₋₁₀)bicycloaryl as used in this Application includes, but is not limited to, 2-amino-4-oxo-3,4-dihydropteridin-6-yl, and the like. In general, the term heterobicycloaryl as used in this Application includes, for example, benzo[1,3]dioxol-5-yl, 3,4-dihydro-2*H*-[1,8]naphthyridinyl, 3,4-dihydro-2*H*-quinolinyl, 2,4-dioxo-3,4-dihydro-2*H*-quinazolinyl, 1,2,3,4,5,6-hexahydro[2,2']bipyridinyl, 3-oxo-2,3-dihydrobenzo[1,4]oxazinyl, 5,6,7,8-tetrahydroquinolinyl, and the like.

[0069] "Heterocycloalkyl" means cycloalkyl, as defined in this Application, provided that one or more of the ring carbon atoms indicated are replaced by a heteroatom.

[0070] "Hydroxy" means the radical -OH.

[0071] "Isomers" mean any compound having an identical molecular formulae but differing in the nature or sequence of bonding of their atoms or in the arrangement of their atoms in space. Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers". Stereoisomers that are not mirror images of one another are termed "diastereomers" and stereoisomers that are nonsuperimposable mirror images are termed "enantiomers" or sometimes "optical isomers". A carbon atom bonded to four nonidentical substituents is termed a "chiral center". A compound with one chiral center has two enantiomeric forms of opposite chirality. A mixture of the two enantiomeric forms is termed a "racemic mixture". A compound that has more than one chiral center has 2^{n-1} enantiomeric pairs, where n is the number of chiral centers. Compounds with more than one chiral center may exist as either an individual diastereomers or as a mixture of diastereomers, termed a "diastereomeric mixture". When one chiral center is present a stereoisomer may be characterized by the absolute configuration of that chiral center. Absolute configuration refers to the arrangement in space of the substituents attached to the chiral center. Enantiomers are characterized by the absolute configuration of their chiral centers and described by the *R*- and *S*-sequencing rules of Cahn, Ingold and Prelog. Conventions for stereochemical nomenclature, methods for the determination of stereochemistry and the separation of stereoisomers are well known in the art (e.g., see "Advanced Organic Chemistry", 4th edition, March, Jerry, John Wiley & Sons, New York, 1992).

[0072] "Oxaalkyl" means an alkyl, as defined above, except where one or more oxygen atoms (-O-) are positioned between carbon atoms of the alkyl. For example, an (C₂₋₆)oxaalkyl refers to a chain comprising between 2 and 6 carbons and one or more oxygen atoms positioned between the carbon atoms.

[0073] "Oxoalkyl" means an alkyl, further substituted with a carbonyl group. The carbonyl group may be an aldehyde, ketone, ester, amide, acid or acid chloride.

[0074] "Pharmaceutically acceptable" means that which is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable and includes that which is acceptable for veterinary use as well as human pharmaceutical use.

[0075] "Pharmaceutically acceptable salts" means salts of inhibitors of the present invention which are pharmaceutically acceptable, as defined above, and which possess the desired pharmacological activity. Such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or with organic acids such as acetic acid, propionic acid, hexanoic acid, heptanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, *o*-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, madelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, *p*-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, *p*-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]oct-2-ene-1-carboxylic acid, glucoheptonic acid, 4,4'-methylenebis(3-hydroxy-2-ene-1-carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid and the like.

[0076] Pharmaceutically acceptable salts also include base addition salts which may be formed when acidic protons present are capable of reacting with inorganic or organic bases. Acceptable inorganic bases include sodium hydroxide, sodium carbonate, potassium hydroxide, aluminum hydroxide and calcium hydroxide. Acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, *N*-methylglucamine and the like.

[0077] "Prodrug" means a compound that is convertible *in vivo* metabolically into an inhibitor according to the present invention. For example an inhibitor comprising a hydroxy group may be administered as an ester that is converted by hydrolysis *in vivo* to the hydroxy compound. Alternatively, an inhibitor comprising a hydroxy group may be administered as an ester and converted by hydrolysis *in vivo* to the hydroxy compound. Suitable esters that may be converted *in vivo* into hydroxy compounds include acetates, citrates, lactates, tartrates, malonates, oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene-bis-*b*-hydroxynaphthoates, gentisates, isethionates, di-*p*-toluoyltartrates, methanesulphonates, ethanesulphonates, benzenesulphonates, *p*-toluenesulphonates, cyclohexylsulphamates and quinates.

[0078] "Protected derivatives" means derivatives of inhibitors in which a reactive site or sites are blocked with protecting groups. Protected derivatives are useful in the preparation of inhibitors or in themselves may be active as inhibitors. A comprehensive list of suitable protecting groups can be found in T.W. Greene, *Protecting Groups in Organic Synthesis*, 3rd edition, John Wiley & Sons, Inc. 1999.

[0079] "Therapeutically effective amount" means that amount which, when administered to an animal for treating a disease, is sufficient to effect such treatment for the disease.

[0080] "Thioketone derivative" means a derivative containing the moiety -C(S)-.

[0081] "Treatment" or "treating" means any administration of a compound of the present invention and includes:

(1) preventing the disease from occurring in an animal which may be predisposed to the disease but does not yet experience or display the pathology or symptomatology of the disease,

(2) inhibiting the disease in an animal that is experiencing or displaying the pathology or symptomatology of the diseased (i.e., arresting further development of the pathology and/or symptomatology), or

(3) ameliorating the disease in an animal that is experiencing or displaying the pathology or symptomatology of the diseased (i.e., reversing the pathology and/or symptomatology).

[0082] It is noted in regard to all of the definitions provided herein that the definitions should be interpreted as being open ended in the sense that further substituents beyond those specified may be included. Hence, a C₁ alkyl indicates that there is one carbon atom but does not indicate what are the substituents on the carbon atom. Hence, a C₁ alkyl comprises methyl (i.e., -CH₃) as well as -C R₁R₂R₃ where R₁, R₂, and R₃ may each independently be hydrogen or any other substituent where the atom alpha to the carbon is a heteroatom. Hence, CF₃, CH₂OH and CH₂CN are all C₁ alkyls.

DETAILED DESCRIPTION OF THE INVENTION

[0083] The present invention relates to compounds, compositions, kits and articles of manufacture that may be used to inhibit histone deacetylases (referred to herein as HDACs). The

compounds may optionally be more particularly used as inhibitors of Class I HDACs, and optionally as inhibitors of HDAC8.

[0084] At least seventeen human genes that encode proven or putative HDACs have been identified to date, some of which are described in Johnstone, R. W., "Histone-Deacetylase Inhibitors: Novel Drugs for the Treatment of Cancer", Nature Reviews, Volume I, pp. 287-299, (2002) and PCT Publication Nos. 00/10583, 01/18045, 01/42437 and 02/08273.

[0085] HDACs have been categorized into three distinct classes based on their relative size and sequence homology. The different HDACs (*Homo sapiens*), HDAC classes, sequences and references describing the different HDACs are provided in Tables 1 - 3.

TABLE 1: CLASS I HDACs

HDAC	GenBank Accession Number	Reference
1	NP_004955	Histone deacetylase: a regulator of transcription, Wolffe, A.P., Science 272 (5260), 371-372 (1996)
2	NP_001518	Isolation and mapping of a human gene (RPD3L1) that is homologous to RPD3, a transcription factor in <i>Saccharomyces cerevisiae</i> ; Furukawa, Y., Kawakami, T., Sudo, K., Inazawa, J., Matsumine, A., Akiyama, T. and Nakamura, Y., Cytogenet. Cell Genet. 73 (1-2), 130-133 (1996)
3	NP_003874	Isolation and characterization of cDNAs corresponding to an additional member of the human histone deacetylase gene family, Yang, W.M., Yao, Y.L., Sun, J.M., Davie, J.R. and Seto, E., J. Biol. Chem. 272 (44), 28001-28007 (1997)
8	NP_060956	Buggy, J.J., Sideris, M.L., Mak, P., Lorimer, D.D., McIntosh, B. and Clark, J.M., Biochem. J. 350 Pt 1, 199-205 (2000)
11	NP_079103	Cloning and Functional Characterization of HDAC11, a Novel Member of the Human Histone Deacetylase Family, Gao, L., Cueto, M.A., Asselbergs, F. and Atadja, P., J. Biol. Chem. 277 (28), 25748-25755 (2002)

TABLE 2: CLASS II HDACs

HDAC	GenBank Accession Number	Reference
4	NP_006028	Transcriptional control. Sinful repression, Wolffe,A.P., Nature 387 (6628), 16-17 (1997)
5	NP_631944	Prediction of the coding sequences of unidentified human genes. IX. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro, Nagase,T., Ishikawa,K., Miyajima,N., Tanaka,A., Kotani,H., Nomura,N. and Ohara,O., DNA Res. 5 (1), 31-39 (1998)
6	NP_006035	Transcriptional control. Sinful repression, Wolffe,A.P., Nature 387 (6628), 16-17 (1997)
7	NP_057680	Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression, Kao,H.Y., Downes,M., Ordentlich,P. and Evans,R.M., Genes Dev. 14 (1), 55-66 (2000)
9	NP_478056	MEF-2 function is modified by a novel co-repressor, MITR, Sparrow,D.B., Miska,E.A., Langley,E., Reynaud-Deonauth,S., Kotecha,S., Towers,N., Spohr,G., Kouzarides,T. and Mohun,T.J., EMBO J. 18 (18), 5085-5098 (1999)
10	NP_114408	Isolation and characterization of mammalian HDAC10, a novel histone deacetylase, Kao,H.Y., Lee,C.H., Komarov,A., Han,C.C. and Evans,R.M., J. Biol. Chem. 277 (1), 187-193 (2002)

TABLE 3: CLASS III HDACs

HDAC	GenBank Accession Number	Reference
Sirtuin 1	NP_036370	Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity; Frye,R.A.; Biochem. Biophys. Res. Commun. 260 (1), 273-279 (1999)
Sirtuin 2	NP_085096 / NP_036369	A 'double adaptor' method for improved shotgun library construction; Andersson,B., Wentland,M.A., Ricafrente,J.Y., Liu,W. and Gibbs,R.A.; Anal. Biochem. 236 (1), 107-113 (1996)

Sirtuin 3	NP_036371	Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity; Frye,R.A.; Biochem. Biophys. Res. Commun. 260 (1), 273-279 (1999)
Sirtuin 4	NP_036372	Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity; Frye,R.A.; Biochem. Biophys. Res. Commun. 260 (1), 273-279 (1999)
Sirtuin 5	NP_112534 / NP_036373	Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity; Frye,R.A.; Biochem. Biophys. Res. Commun. 260 (1), 273-279 (1999)
Sirtuin 6	NP_057623	Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins; Frye,R.A.; Biochem. Biophys. Res. Commun. 273 (2), 793-798 (2000)
Sirtuin 7	NP_057622	Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins; Frye,R.A.; Biochem. Biophys. Res. Commun. 273 (2), 793-798 (2000)

[0086] Of particular note are Class I HDACs. All Class I HDACs appear to be sensitive to inhibition by trichostatin A (TSA). Also of particular note is HDAC8, a protein whose crystal structure Applicants recently determined.

[0087] HDAC8 is a 377 residue, 42kDa protein localized to the nucleus of a wide array of tissues, as well as several human tumor cell lines. The wild-type form of full length HDAC8 is described in GenBank Accession Number NP 060956; Buggy,J.J., Sideris,M.L., Mak,P., Lorimer,D.D., McIntosh,B. and Clark,J.M., Cloning and characterization of a novel human histone deacetylase, HDAC8, Biochem. J. 350 Pt 1, 199-205 (2000). Zn²⁺ is likely native to the protein and required for HDAC8 activity.

1. CRYSTAL STRUCTURE FOR HDAC

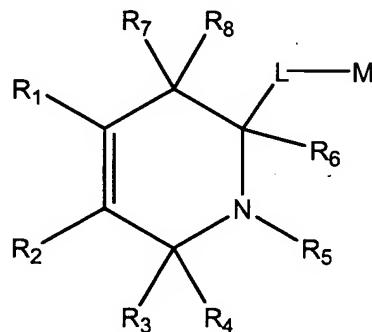
[0088] Syrrx, Inc. in San Diego, California recently solved the crystal structure for HDAC8. Knowledge of the crystal structure was used to guide the design of the HDAC inhibitors provided herein.

[0089] Figure 1 illustrates a ribbon diagram overview of the structure of HDAC8, highlighting the secondary structural elements of the protein. HDAC8 was found to have a single

domain structure belonging to the open α/β class of folds. The structure consists of a central 8-stranded parallel β -sheet sandwiched between layers of α -helices. The ligand binding clefts lie almost in the plane of the central β -sheet, and are formed primarily by loops emanating from the carboxy-terminal ends of the β -strands comprising the sheet. There are two large structural extensions, which occur beyond the core of the α/β motif, off the second and last β -strands of the central β -sheet. Residues contained in the extension off the second β -strand form a globular "cap" over the core of the protein, play an important role in defining the shape of the ligand binding pockets, and are involved in a number of key interactions with the bound ligands.

2. HDAC INHIBITORS

[0090] In one embodiment, HDAC inhibitors of the present invention are provided that comprise the formula



wherein

R₁ and R₂ are taken together to form a substituted or unsubstituted aromatic ring;

R₃ and R₄ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, and a thiocarbonyl group or where R₃ and R₄ are taken together to form a ring;

R₅ is selected from a group of substituents that comprise a moiety attached to the ring nitrogen selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R₆ is selected from a group of substituents that comprise a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R₇ and R₈ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonlamino, nitro, cyano, halogen, hydroxyl, thiol, amino, a carbonyl group, and a thiocarbonyl group, or R₇ and R₈ are taken together to form a substituent comprising a moiety attached to the ring carbon selected from the group consisting of a carbonyl, thiocarbonyl, imine, alkene and ring;

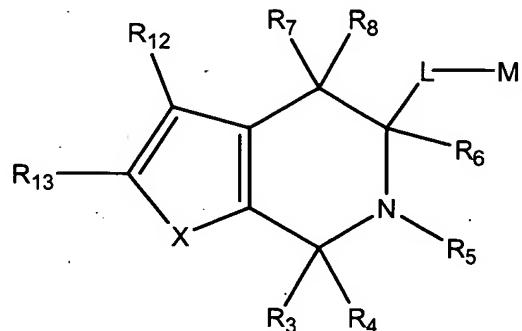
M is a substituent capable of complexing with a protein metal ion; and

L is a substituent comprising a chain of 3-12 atoms connecting the M substituent to the carbon atom alpha to the L substituent.

R₁ and R₂

[0091] In regard to each of the above embodiments, R₁ and R₂ may optionally be selected such that the substituted or unsubstituted aromatic ring formed when R₁ and R₂ are taken together is a substituted or unsubstituted aryl ring. R₁ and R₂ may also optionally be selected such that an aryl ring is formed that is substituted and comprises one or more substituents that together form a ring fused to the aryl ring. R₁ and R₂ may also optionally be selected such that an aryl ring is formed that is a substituted or unsubstituted phenyl ring. The phenyl ring may optionally be substituted and comprise one or more substituents that together form a ring fused to the phenyl ring. R₁ and R₂ may also optionally be selected such that an aryl ring is formed that is a substituted or unsubstituted heteroaryl ring. For example, the substituted or unsubstituted heteroaryl ring may optionally be selected from the group of substituted or unsubstituted heteroaryl rings consisting of furan, thifuran, pyrrole, pyrazole, imidazole, triazole, isoxazole, oxazole, thiazole, isothiazole, pyridine, pyridazine, pyrimidine, pyrazine, benzofuran, benzothifuran, indole, quinoline, isoquinoline, cinnoline, naphthyridine, and pyridopyridine. The heteroaryl ring may also comprise one or more substituents that together form a ring fused to the heteroaryl ring.

[0092] In one variation where R₁ and R₂ are taken together to form a five membered heteroaryl ring, HDAC inhibitors are provided that comprise the formula



wherein

R₃ and R₄ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, and a thiocarbonyl group or where R₃ and R₄ are taken together to form a ring;

R₅ is selected from a group of substituents that comprise a moiety attached to the ring nitrogen selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R₆ is selected from a group of substituents that comprise a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R₇ and R₈ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonylamino, nitro, cyano, halogen, hydroxyl, thiol, amino, a carbonyl group, and a thiocarbonyl group, or R₇ and R₈ are taken together to form a substituent comprising a moiety attached to the ring carbon selected from the group consisting of a carbonyl, thiocarbonyl, imine, alkene and ring;

R₁₂ and R₁₃ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl,

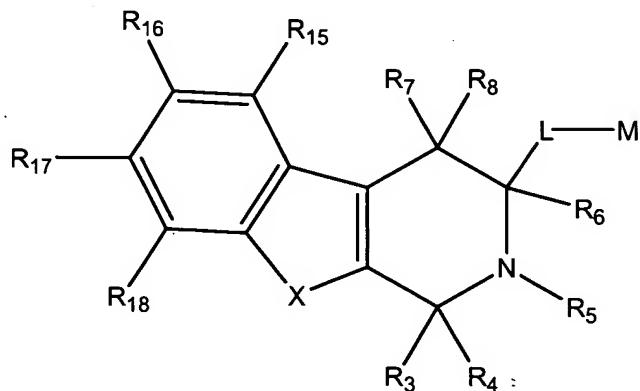
oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonylamino, nitro, cyano, halogen, hydroxyl, thiol, amino, a carbonyl group, and a thiocarbonyl group, or R₇ and R₈ are taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring;

X is selected from the group consisting of O, S, and NR₁₄, where R₁₄ comprises a moiety attached to the nitrogen selected from the group consisting of hydrogen, hydroxyl, alkyl, aromatic ring, alkoxy, aryloxy, a carbonyl group, a thiocarbonyl group, and a sulfonyl group;

M is a substituent capable of complexing with a protein metal ion; and

L is a substituent comprising a chain of 3-12 atoms connecting the M substituent to the carbon atom alpha to the L substituent.

[0093] In another variation where R₁ and R₂ are taken together to form a five membered heteroaryl ring, HDAC inhibitors are provided that comprise the formula



wherein

R₃ and R₄ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, and a thiocarbonyl group or where R₃ and R₄ are taken together to form a ring;

R₅ is selected from a group of substituents that comprise a moiety attached to the ring nitrogen selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R_6 is selected from a group of substituents that comprise a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R_7 and R_8 are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonylamino, nitro, cyano, halogen, hydroxyl, thiol, amino, a carbonyl group, and a thiocarbonyl group, or R_7 and R_8 are taken together to form a substituent comprising a moiety attached to the ring carbon selected from the group consisting of a carbonyl, thiocarbonyl, imine, alkene and ring;

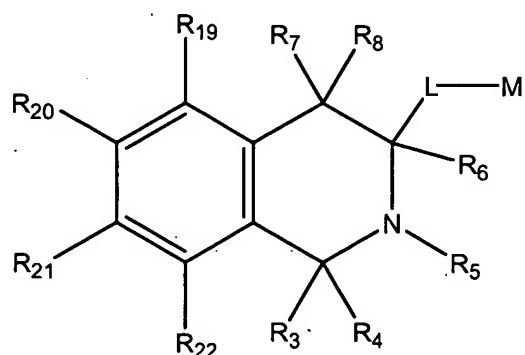
R_{15} , R_{16} , R_{17} and R_{18} are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonylamino, nitro, cyano, halogen, hydroxyl, thiol, amino, a carbonyl group, and a thiocarbonyl group, except where R_{15} and R_{16} , R_{16} and R_{17} , and/or R_{17} and R_{18} are taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring;

X is selected from the group consisting of O, S, and NR_{14} , where R_{14} comprises a moiety attached to the nitrogen selected from the group consisting of hydrogen, hydroxyl, alkyl, aromatic ring, alkoxy, aryloxy, a carbonyl group, a thiocarbonyl group, and a sulfonyl group;

M is a substituent capable of complexing with a protein metal ion; and

L is a substituent comprising a chain of 3-12 atoms connecting the M substituent to the carbon atom alpha to the L substituent.

[0094] In one variation where R_1 and R_2 are taken together to form a six membered aryl ring, HDAC inhibitors are provided that comprise the formula



wherein

R₃ and R₄ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, and a thiocarbonyl group or where R₃ and R₄ are taken together to form a ring;

R₅ is selected from a group of substituents that comprise a moiety attached to the ring nitrogen selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R₆ is selected from a group of substituents that comprise a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, cyano, a carbonyl group, a thiocarbonyl group and a sulfonyl group;

R₇ and R₈ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonylamino, nitro, cyano, halogen, hydroxyl, thiol, amino, a carbonyl group, and a thiocarbonyl group, or R₇ and R₈ are taken together to form a substituent comprising a moiety attached to the ring carbon selected from the group consisting of a carbonyl, thiocarbonyl, imine, alkene and ring;

R₁₉, R₂₀, R₂₁ and R₂₂ are each independently selected from a group of substituents comprising a moiety attached to the ring carbon selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonylamino, nitro, cyano, halogen, hydroxyl, thiol, amino, a carbonyl group, and a thiocarbonyl group, except where R₁₉ and R₂₀, R₂₀ and R₂₁, and/or R₂₁ and R₂₂ are taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring;

X is selected from the group consisting of O, S, and NR₁₄, where R₁₄ comprises a moiety attached to the nitrogen selected from the group consisting of hydrogen, hydroxyl, alkyl, aromatic ring, alkoxy, aryloxy, a carbonyl group, a thiocarbonyl group, and a sulfonyl group;

M is a substituent capable of complexing with a protein metal ion; and

L is a substituent comprising a chain of 3-12 atoms connecting the M substituent to the carbon atom alpha to the L substituent.

R₃ and R₄

[0095] Also in regard to each of the above embodiments, at least one of R₃ and R₄ may optionally be selected from a group of substituents where the moiety attached to the ring carbon is a C₁-C₁₀ alkyl, aminoalkyl, or oxaalkyl. R₃ and R₄ may also optionally be selected from a group of substituents where the moiety attached to the ring carbon is a branched C₁-C₁₀ alkyl, aminoalkyl, or oxaalkyl. The C₁-C₁₀ alkyl, aminoalkyl, or oxaalkyl may optionally further comprise a substituent selected from the group consisting of an alkyl, aromatic ring, cyano group, halogen, and carbonyl group. The C₁-C₁₀ alkyl, aminoalkyl, or oxaalkyl may also optionally further comprise a substituted or unsubstituted aromatic ring.

[0096] Also in regard to each of the above embodiments, at least one of R₃ and R₄ may optionally be selected from a group of substituents where the moiety attached to the ring carbon is an aromatic ring.

[0097] Also in regard to each of the above embodiments, at least one of R₃ and R₄ may optionally be selected from a group of substituents where the moiety attached to the ring carbon is a substituted or unsubstituted phenyl ring.

[0098] Also in regard to each of the above embodiments, at least one of R₃ and R₄ may optionally be selected from a group of substituents where the moiety attached to the ring carbon is a substituted or unsubstituted heteroaryl.

[0099] Also in regard to each of the above embodiments, at least one of R₃ and R₄ may optionally be selected from a group of substituents where the moiety attached to the ring carbon is a substituted or unsubstituted heteroaryl selected from the group consisting of furan, thifuran, pyrrole, pyrazole, isoimidazole, triazole, isoxazole, oxazole, thiazole, isothiazole, oxadiazole, oxatriazole, pyridine, pyridazine, pyrimidine, pyrazine, triazine, benzofuran, isobenzofuran, benzothifuran, isobenzothifuran, indole, isobenzazole, quinoline, isoquinoline, cinnoline, quinazoline, naphthyridine, and pyridopyridine.

[0100] Also in regard to each of the above embodiments, R₃ and R₄ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring.

[0101] Also in regard to each of the above embodiments, R₃ and R₄ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 alicyclic ring.

[00102] Also in regard to each of the above embodiments, at least one of R₃ and R₄ may optionally be selected from a group of substituents where the moiety attached to the ring carbon is selected from the group consisting of an aldehyde, amide, ester, ketone, and carboxylic acid.

R₅ and R₆

[00103] In regard to each of the above embodiments, R₅ and R₆ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring.

[00104] Also in regard to each of the above embodiments, R₅ and R₆ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 alicyclic ring.

R₆ and R₇

[00105] In regard to each of the above embodiments, R₆ and R₇ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring.

[00106] Also in regard to each of the above embodiments, R₆ and R₇ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 alicyclic ring.

R₇ and R₈

[00107] In regard to each of the above embodiments, R₇ and R₈ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring.

[00108] Also in regard to each of the above embodiments, R₇ and R₈ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 alicyclic ring.

[00109] Also in regard to each of the above embodiments, R₇ and R₈ may optionally be taken together to form an imine having a substituent R₉ on the imine nitrogen selected from the group consisting of hydrogen, alkyl, aminoalkyl, oxaalkyl, aromatic ring, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, and sulfonylamino.

[00110] Also in regard to each of the above embodiments, R₇ and R₈ may optionally be taken together to form an alkene substituent having the formula =CR₁₀R₁₁ where R₁₀ and R₁₁ are each independently selected from a group of substituents consisting of hydrogen, halogen, alkyl, aryl, alkoxy, aryloxy, alkylamino, arylamino, alkylthio, arylthio, acylamino, sulfonylamino,

cyano, nitro, a carbonyl group, thiocarbonyl, and sulfonyl or where R₁₀ and R₁₁ are taken together to form an alkene.

[00111] Also in regard to each of the above embodiments, R₇ and R₈ may optionally be taken together to form an alkene substituent having the formula =CR₁₀R₁₁ where R₁₀ and R₁₁ are together together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 membered ring. R₁₀ and R₁₁ may optionally be taken together to form a substituted or unsubstituted 3, 4, 5, 6, 7 or 8 member alicyclic ring.

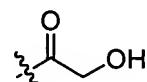
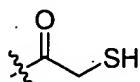
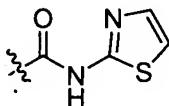
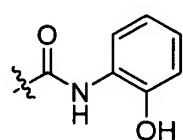
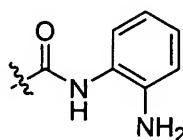
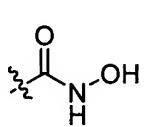
[00112] In regard to each of the above embodiments, it is noted that alkyl may be selected from the group consisting of a straight chained alkyl, branched alkyl, cycloalkyl, arylalkyl, heteroarylalkyl, oxoalkyl, halogen substituted alkyl, aminoalkyl, sulfonamide alkyl.

[00113] Particular examples of scaffold structures that may be formed based on the different R₁, R₂, R₃, R₄, and R₅ are illustrated in Figure 2A.

Metal Ion Complexing Substituent, M

[00114] In regard to each of the above embodiments, substituent M may be any substituent that is capable of complexing with a metal ion, and optionally more particularly a zinc ion since a zinc ion is known to be present in the catalytic site of histone deacetylases. Hence, the M substituent may facilitate inhibitor binding by complexing with the zinc ion present in the catalytic site of histone deacetylases.

[00115] Examples of substituents capable of complexing with a zinc ion that may be used as the M substituent include, but are not limited to trifluoroacetyl (-C(O)-CF₃), -NH-P(O)OH-CH₃, sulfonamides (-SO₂NH₂), thiols(-SH), and carbonyl groups having the formula -C(O)-R₂₅ wherein R₂₅ is hydroxylamino, hydroxyl, amino, alkylamino, or an alkyloxy group. Particular examples of such substituents include:



[00116] In one particular variation, M is a hydroxamic acid (-C(O)-NHOH), also shown above. It is noted that hydroxamic acids, such as trichostatin A, have been shown to be effective inhibitors against histone deacetylases by complexing with the zinc ion present in the catalytic site of histone deacetylases.

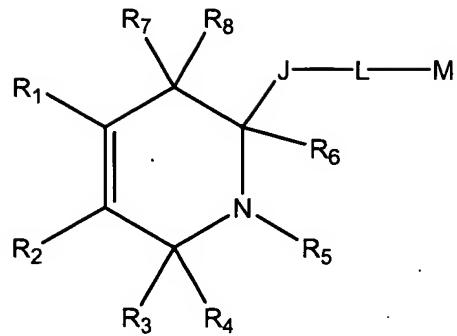
Leader group, L

[00117] In regard to each of the above embodiments, the leader group, L, may be any substituent comprising a chain of 3-12 atoms connecting the M substituent to the carbon atom alpha to the L substituent. The number of atoms in the chain serves to extend the zinc complexing substituent, M, a sufficient distance away from the remainder of the inhibitor so as to allow the zinc complexing substituent to interact with the zinc ion while the remainder of the inhibitor interacts with hydrophobic regions in the binding pocket of the histone deacetylase.

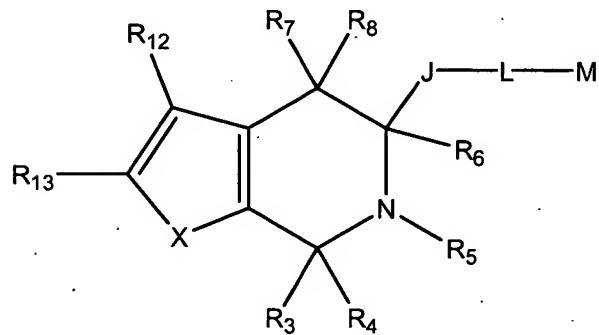
[00118] It is noted that the leader group may comprise a variety of moieties, J, that may be used to link the leader group to the remainder of the compound. Synthetic schemes for using these different J moieties to couple the leader group are provided in the examples. Beyond these schemes, the particular J moiety may be widely varied, depending on the chemistry used to link the leader group to the ring.

[00119] Figure 2B illustrates a variety of J moieties that the leader groups may optionally comprise. In regard to substituent R₂₃ shown in Figure 2B, the substituent may optionally be a C₁₋₁₀ alkyl.

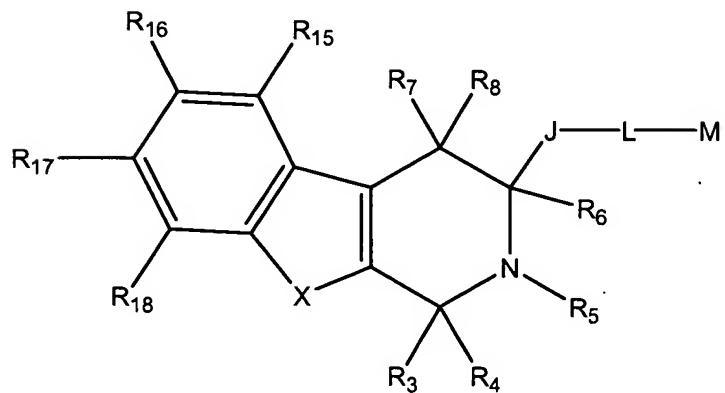
[00120] When, the leader group comprises the J moiety, the compounds according to the present invention may be expressed as comprising the below formulas where the leader group L comprises moieties as is otherwise described herein.



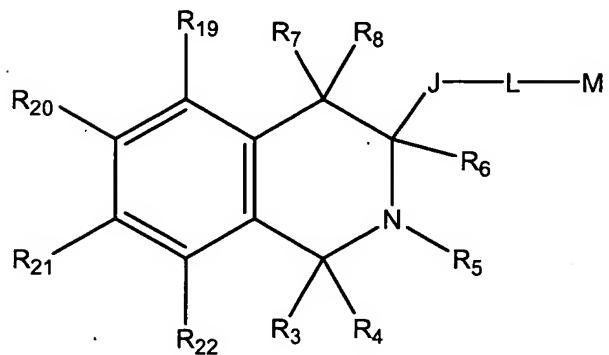
or



or



or



where the other substituents may be as otherwise described herein.

[00121] In one embodiment, the leader group, L, comprises a chain of 3-12 atoms that extend between the carbon alpha to the leader group and the M substituent, optionally 3-9 and optionally 4-8 atoms. In one variation, the number of atoms in the chain of atoms extending between the carbon alpha to the leader group and the M substituent is 3, 4, 5, 6, 7, 8 or 9 atoms.

[00122] It is noted that the chain of atoms extending between the carbon alpha to the leader group and the M substituent may consist only of carbon atoms. Alternatively, the chain may also comprise non-carbon atoms such as nitrogen, oxygen and sulphur.

[00123] It is also noted that the bonds forming the chain of atoms extending between the carbon alpha to the leader group and the M substituent may be saturated, partially unsaturated, or fully unsaturated. For example, the leader group may comprise as part of the chain of atoms one or more alkene (-CH=CH-) or alkyne (-C≡C-) bonds.

[00124] A variety of different moieties may be incorporated into the leader groups of the HDAC inhibitors of the present invention. Examples of such moieties are shown in Figure 2C. It is noted that the moieties may be incorporated with or without the J moiety and may be incorporated in either orientation shown in the figure.

CH₂CH₂CH₂CHCH=CH-; -C(CH₃)=CH- and -CH=C(CH₃)-; -C(CH₃)=CHCH₂-, -CH=C(CH₃)CH₂-, and -CH=CHCH(CH₃)-; -CH(CH₃)CH=CH-, -CH₂C(CH₃)=CH-, and -CH₂CH=C(CH₃)-; -CH=CHCH=CH-; -CH=CHCH=CHCH₂-, -CH₂CH=CHCH=CH-, and -CH=CHCH₂CH=CH-; -CH=CHCH=CHCH₂CH₂-, -CH=CHCH₂CH=CHCH₂-, and -CH=CHCH₂CH₂CH=CH-, -CH₂CH=CHCH=CHCH₂-, -CH₂CH=CHCH₂CH=CH-, and -CH₂CH₂CH=CHCH=CH-; -C(CH₃)=CHCH=CH -, -CH=C(CH₃)CH =CH -, -CH=CHC(CH₃)=CH -, and -CH=CHCH=C(CH₃)- ; -C≡C-; -C≡CCH₂-, -CH₂C≡C- ; -C≡CCH(CH₃)- , and -CH(CH₃)C≡C- ; -C=CCH₂CH₂-, -CH₂C-CCH₂-, and -CH₂CH₂C=C-; -C≡CCH(CH₃)CH₂- and -C≡CCH₂CH(CH₃)-; -CH(CH₃)C=CCH₂- and -CH₂C≡CCH(CH₃)-; -CH(CH₃)CH₂C≡C- and -CH₂CH(CH₃)C≡C-; -C≡CCH=CH-, -CH=CHC≡C-, and -C≡CC≡C-; -C≡CCH₂CH₂CH₂- and -CH₂CH₂CH₂C≡C-; -C≡CCH₂CH₂CH₂CH₂- and -CH₂CH₂CH₂CH₂C≡C-; -C≡CCH=CHCH=CH-, -CH=CHC≡C-CH=CH-, and -CH=CHCH=CHC≡C-; -C(CH₃)=CHC≡C-, -CH=C(CH₃)C≡C-, - C≡CC(CH₃)=CH- , and -C≡CCH=C(CH₃)-. It is noted that the hydrogen atoms of above possible portions of the leader group may optionally be substituted with further substituents.

[00126] It is also noted that the leader group may comprise one or more substituents extending from one or more atoms of the leader group backbone. In one variation, two substituents extending from the atoms extending between the carbon alpha to the leader group and the M substituent to form one or more three, four, five, six, seven, eight or nine membered rings. The atoms of the leader group forming the ring may be separated from each other by 0, 1, 2, 3, or 4 atoms.

[00127] The rings may be saturated or partially unsaturated (i.e., comprise one or two double bonds). The rings may also be aromatic, referred to herein as aryl and heteroaryl rings. The rings may optionally be further substituted. These further ring substituents may combine to form additional rings that are fused to the rings forming a portion of the backbone, e.g., bicycloaryl and bicycloheteroaryl.

[00128] Examples of cycloalkyl rings that may be formed by one or more leader group backbone atoms include, but are not limited to: cyclopropyl, cyclohexane, cyclopentane, cyclopentene, cyclopentadiene, cyclohexane, cyclohexene, cyclohexadiene, phenyl, cycloheptane, cycloheptene, cycloheptadiene, cyclooctane, cyclooctene, and cyclooctadiene.

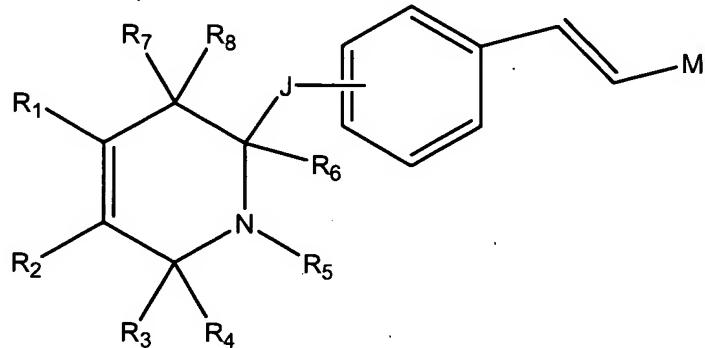
[00129] Examples of aryl rings that may be formed by one or more leader group backbone atoms include, but are not limited to: phenyl, biphenyl-2-yl, 2-bromophenyl, 2-bromocarbonylphenyl, 2-bromo-5-fluorophenyl, 4-*tert*-butylphenyl, 4-carbamoylphenyl, 4-carboxy-2-nitrophenyl, 2-chlorophenyl, 4-chlorophenyl, 3-chlorocarbonylphenyl, 4-chlorocarbonylphenyl, 2-chloro-4-fluorophenyl, 2-chloro-6-fluorophenyl, 4-chloro-2-nitrophenyl, 6-chloro-2-nitrophenyl, 2,6-dibromophenyl, 2,3-dichlorophenyl, 2,5-dichlorophenyl, 3,4-dichlorophenyl, 2-difluoromethoxyphenyl, 3,5-dimethylphenyl, 2-ethoxycarbonylphenyl, 2-fluorophenyl, 2-iodophenyl, 4-isopropylphenyl, 2-methoxyphenyl, 4-methoxyphenyl, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 5-methyl-2-nitrophenyl, 4-methylsulfonylphenyl, naphth-2-yl, 2-nitrophenyl, 3-nitrophenyl, 4-nitrophenyl, 2,3,4,5,6-pentafluorophenyl, phenyl, 2-trifluoromethoxyphenyl, 3-trifluoromethoxyphenyl, 4-trifluoromethoxyphenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 2-trifluoromethylsulfanylphenyl, 4-trifluoromethylsulfanylphenyl, and the like.

[00130] Examples of heteroaryl rings that may be formed by one or more leader group backbone atoms include, but are not limited to: furan, thifuran, pyrrole, isopyrrole, 3-isopyrrole, pyrazole, isoimidazole, triazole, isoxazole, oxazole, thiazole, isothiazole, oxadiazole, oxatriazole, pyridine, pyridazine, pyrimidine, pyrazine, triazine, benzofuran, isobenzofuran, benzothifuran, isobenzothifuran, indole, isobenzazole, quinoline, isoquinoline, cinnoline, quinazoline, naphthyridine, and pyridopyridine

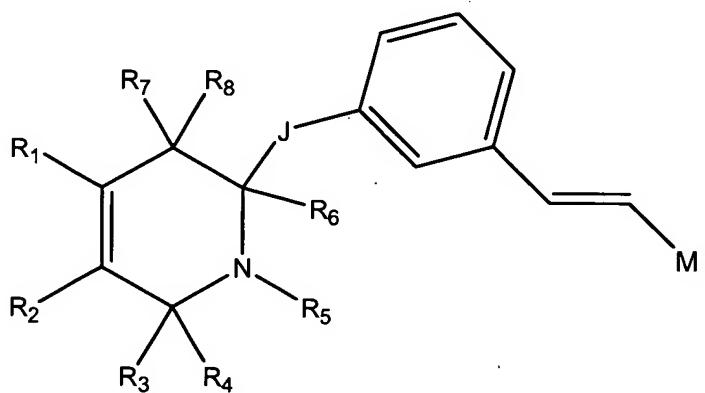
[00131] Particular examples of heteroaryl rings that may be formed by one or more leader group backbone atoms include, but are not limited to: 4-amino-2-hydroxypyrimidin-5-yl, dibenzofuranyl, benzothiazol-2-yl, 1*H*-benzoimidazol-2-yl, 2-bromopyrid-5-yl, 5-bromopyrid-2-yl, 4-carbamoylthiazol-2-yl, 3-carboxypyrid-4-yl, 5-carboxy-2,6-dimethylpyrid-3-yl, 3,5-dimethylisoxazol-4-yl, 5-ethoxy-2,6-dimethylpyrid-3-yl, 5-fluoro-6-hydroxypyrimidin-4-yl, fur-2-yl, fur-3-yl, 5-hydroxy-4,6-dimethylpyrid-3-yl, 8-hydroxy-5,7-dimethylquinolin-2-yl, 5-hydroxymethylisoxazol-3-yl, 3-hydroxy-6-methylpyrid-2-yl, 3-hydroxypyrid-2-yl, 1*H*-imidazol-2-yl, 1*H*-imidazol-4-yl, 1*H*-indol-3-yl, isothiazol-4-yl, isoxazol-4-yl, 2-methylfur-3-yl, 5-methylfur-2-yl, 1-methyl-1*H*-imidazol-2-yl, 5-methyl-3*H*-imidazol-4-yl, 5-methylisoxazol-3-yl, 5-methyl-2*H*-pyrazol-3-yl, 3-methylpyrid-2-yl,

4-methylpyrid-2-yl, 5-methylpyrid-2-yl, 6-methylpyrid-2-yl, 2-methylpyrid-3-yl, 2-methylthiazol-4-yl, 5-nitropyrid-2-yl, 2*H*-pyrazol-3-yl, 3*H*-pyrazol-4-yl, pyridazin-3-yl, pyrid-2-yl, pyrid-3-yl, pyrid-4-yl, 5-pyrid-3-yl-2*H*-[1,2,4]triazol-3-yl, pyrimidin-4-yl, pyrimidin-5-yl, 1*H*-pyrrol-3-yl, quinolin-2-yl, 1*H*-tetrazol-5-yl, thiazol-2-yl, thiazol-5-yl, thien-2-yl, thien-3-yl, 2*H*-[1,2,4]triazol-3-yl, 3*H*-[1,2,3]triazol-4-yl, 5-trifluoromethylpyrid-2-yl, and the like. Suitable protecting groups include *tert*-butoxycarbonyl, benzyloxycarbonyl, benzyl, 4-methoxybenzyl, 2-nitrobenzyl, and the like.

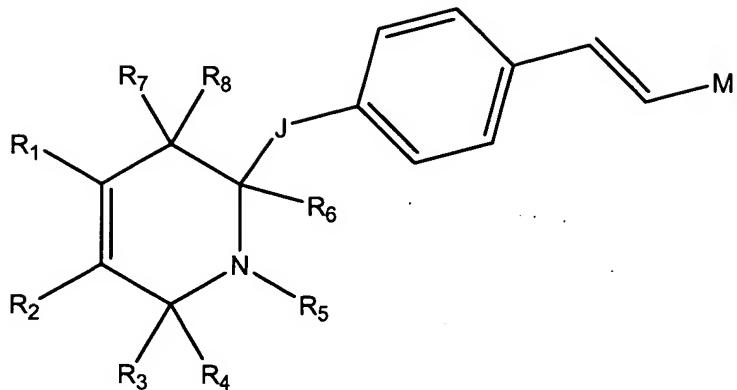
[00132] In one particular variation, the ring formed by the backbone of the leader group is a six membered ring, such as a phenyl ring. Examples of such leader groups include meta and para cinnamate. In one embodiment where the leader group is cinnamate, HDAC inhibitors of the present invention are provided that comprise the formula



wherein the alkene is attached to the ring at the meta or para position. It is noted that the other substituents may be as otherwise described herein. For example, in one variation, the HDAC inhibitors have the formula

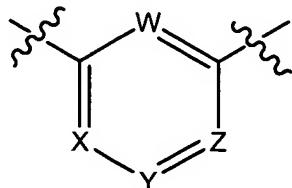


and in another variation has the formula

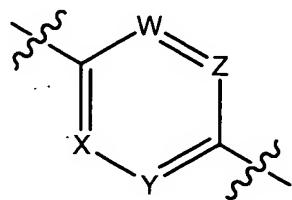


where the other substituents may be as otherwise described herein.

[00133] In another particular variation, the ring comprises heteroatoms. Examples of such rings comprising heteroatoms, including 5 and 6 membered aromatic rings comprising heteroatoms are illustrated in Figure 2C. Optionally, the chain atoms of the leader group are meta or para substituents of the heteroaryl ring. For example, the heteroaryl ring optionally has the formula

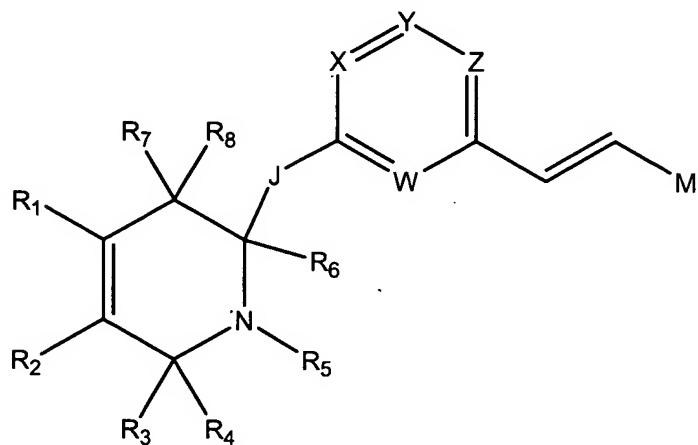


or

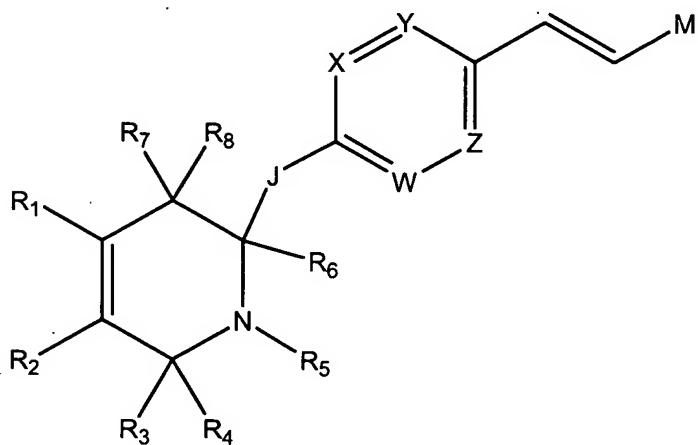


where W, X, Y, and Z are each independently N or CH, with a proviso that when X and Z are both N, then Y is CH.

[00134] In one variation where the leader group comprises the above heteroaryl rings and an alkene, HDAC inhibitors of the present invention are provided that comprise the formula



or



where W, X, Y, and Z are each independently N or CH, with a proviso that when X and Z are both N, then Y is CH; and

where the other substituents may be as otherwise described herein.

[00135] It is noted that the inhibitors may include one or more chiral centers. The chiral centers may be either the R or S enantiomers, depending on the substituents.

[00136] Synthetic scheme for synthesizing compounds according to these various embodiments are provided in the Examples. Particular examples of HDAC inhibitors according to these embodiments are provided in the examples in Figure 2D.

A. Salts, Hydrates, and Prodrugs of HDAC Inhibitors

[00137] It should be recognized that the compounds of the present invention may be present and optionally administered in the form of salts, hydrates and prodrugs that are converted in vivo into the compounds of the present invention. For example, it is within the scope of the present invention to convert the compounds of the present invention into and use them in the form of their pharmaceutically acceptable salts derived from various organic and inorganic acids and bases in accordance with procedures well known in the art.

[00138] When the compounds of the present invention possess a free base form, the compounds can be prepared as a pharmaceutically acceptable acid addition salt by reacting the free base form of the compound with a pharmaceutically acceptable inorganic or organic acid, e.g., hydrohalides such as hydrochloride, hydrobromide, hydroiodide; other mineral acids and their corresponding salts such as sulfate, nitrate, phosphate, etc.; and alkyl- and monoarylsulfonates such as ethanesulfonate, toluenesulfonate and benzenesulfonate; and other organic acids and their corresponding salts such as acetate, tartrate, maleate, succinate, citrate, benzoate, salicylate and ascorbate. Further acid addition salts of the present invention include, but are not limited to: adipate, alginate, arginate, aspartate, benzenesulfonate (besylate), bisulfate, bisulfite, bromide, butyrate, camphorate, camphorsulfonate, caprylate, chloride, chlorobenzoate, cyclopentanepropionate, digluconate, dihydrogenphosphate, dinitrobenzoate, dodecylsulfate, ethanesulfonate, fumarate, galacterate (from mucic acid), galacturonate, glucoheptaoate, gluconate, glutamate, glycerophosphate, hemisuccinate, hemisulfate, heptanoate, hexanoate, hippurate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, iodide, isethionate, iso-butyrate, lactate, lactobionate, malate, malonate, mandelate, metaphosphate, methanesulfonate, methylbenzoate, monohydrogenphosphate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, oleate, pamoate, pectinate, persulfate, phenylacetate, 3-phenylpropionate, phosphate, phosphonate and phthalate. It should be recognized that the free acid forms will typically differ from their respective salt forms somewhat in physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid forms for the purposes of the present invention.

[00139] When the compounds of the present invention possess a free base form, a pharmaceutically acceptable base addition salt can be prepared by reacting the free acid form of the compound with a pharmaceutically acceptable inorganic or organic base. Examples of such bases are alkali metal hydroxides including potassium, sodium and lithium hydroxides; alkaline earth metal hydroxides such as barium and calcium hydroxides; alkali metal alkoxides, e.g. potassium ethanolate and sodium propanolate; and various organic bases such as ammonium hydroxide, piperidine, diethanolamine and N-methylglutamine. Also included are the aluminium salts of the compounds of the present invention. Further base salts of the present invention include, but are not limited to: copper, ferric, ferrous, lithium, magnesium, manganic, manganous, potassium, sodium and zinc salts. Organic base salts include, but are not limited to, salts of primary, secondary and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, e.g., arginine, betaine, caffeine, chloroprocaine, choline, N, N'-dibenzylethylenediamine (benzathine), dicyclohexylamine, diethanolamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, iso-propylamine, lidocaine, lysine, meglumine, N-methyl-D-glucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethanolamine, triethylamine, trimethylamine, tripropylamine and tris-(hydroxymethyl)-methylamine (tromethamine). It should be recognized that the free base forms will typically differ from their respective salt forms somewhat in physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base forms for the purposes of the present invention.

[00140] Compounds of the present invention, which comprise basic nitrogen-containing groups, may be quaternized with such agents as (C₁₋₄) alkyl halides, e.g., methyl, ethyl, iso-propyl and tert-butyl chlorides, bromides and iodides; di (C₁₋₄) alkyl sulfates, e.g., dimethyl, diethyl and diamyl sulfates; (C₁₀₋₁₈) alkyl halides, e.g., decyl, dodecyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aryl (C₁₋₄) alkyl halides, e.g., benzyl chloride and phenethyl bromide. Such salts permit the preparation of both water-soluble and oil-soluble compounds of the present invention.

[00141] *N*-oxides of compounds according to the present invention can be prepared by methods known to those of ordinary skill in the art. For example, *N*-oxides can be prepared by treating an unoxidized form of the compound with an oxidizing agent (e.g., trifluoroperacetic acid, permaleic acid, perbenzoic acid, peracetic acid, *meta*-chloroperoxybenzoic acid, or the like) in a suitable inert organic solvent (e.g., a halogenated hydrocarbon such as dichloromethane) at approximately 0°C. Alternatively, the *N*-oxides of the compounds can be prepared from the *N*-oxide of an appropriate starting material.

[00142] Prodrug derivatives of compounds according to the present invention can be prepared by modifying substituents of compounds of the present invention that are then converted *in vivo* to a different substituent. It is noted that in many instances, the prodrugs themselves also fall within the scope of the range of compounds according to the present invention. For example, prodrugs can be prepared by reacting a compound with a carbamylating agent (e.g., 1,1-acyloxyalkylcarbonochloridate, *para*-nitrophenyl carbonate, or the like) or an acylating agent. Further examples of methods of making prodrugs are described in Saulnier *et al.*(1994), *Bioorganic and Medicinal Chemistry Letters*, Vol. 4, p. 1985.

[00143] Protected derivatives of compounds of the present invention can also be made. Examples of techniques applicable to the creation of protecting groups and their removal can be found in T.W. Greene, *Protecting Groups in Organic Synthesis*, 3rd edition, John Wiley & Sons, Inc. 1999.

[00144] Compounds of the present invention may also be conveniently prepared, or formed during the process of the invention, as solvates (e.g. hydrates). Hydrates of compounds of the present invention may be conveniently prepared by recrystallization from an aqueous/organic solvent mixture, using organic solvents such as dioxin, tetrahydrofuran or methanol.

[00145] A “pharmaceutically acceptable salt”, as used herein, is intended to encompass any compound according to the present invention that is utilized in the form of a salt thereof, especially where the salt confers on the compound improved pharmacokinetic properties as compared to the free form of compound or a different salt form of the compound. The pharmaceutically acceptable salt form may also initially confer desirable pharmacokinetic properties on the compound that it did not previously possess, and may even positively affect the

pharmacodynamics of the compound with respect to its therapeutic activity in the body. An example of a pharmacokinetic property that may be favorably affected is the manner in which the compound is transported across cell membranes, which in turn may directly and positively affect the absorption, distribution, biotransformation and excretion of the compound. While the route of administration of the pharmaceutical composition is important, and various anatomical, physiological and pathological factors can critically affect bioavailability, the solubility of the compound is usually dependent upon the character of the particular salt form thereof, which it utilized. One of skill in the art will appreciate that an aqueous solution of the compound will provide the most rapid absorption of the compound into the body of a subject being treated, while lipid solutions and suspensions, as well as solid dosage forms, will result in less rapid adsorption of the compound.

3. PREPARATION OF HDAC INHIBITORS

[00146] Various methods may be developed for synthesizing compounds according to the present invention. Representative methods for synthesizing these compounds are provided in the Examples. It is noted, however, that the compounds of the present invention may also be synthesized by other synthetic routes that others may devise.

[00147] It will be readily recognized that certain compounds according to the present invention have atoms with linkages to other atoms that confer a particular stereochemistry to the compound (e.g., chiral centers). It is recognized that synthesis of compounds according to the present invention may result in the creation of mixtures of different stereoisomers (enantiomers, diastereomers). Unless a particular stereochemistry is specified, recitation of a compound is intended to encompass all of the different possible stereoisomers.

[00148] Various methods for separating mixtures of different stereoisomers are known in the art. For example, a racemic mixture of a compound may be reacted with an optically active resolving agent to form a pair of diastereoisomeric compounds. The diastereomers may then be separated in order to recover the optically pure enantiomers. Dissociable complexes may also be used to resolve enantiomers (e.g., crystalline diastereoisomeric salts). Diastereomers typically have sufficiently distinct physical properties (e.g., melting points, boiling points, solubilities, reactivity, etc.) that they can be readily separated by taking advantage of these dissimilarities.

For example, diastereomers can typically be separated by chromatography or by separation/resolution techniques based upon differences in solubility. A more detailed description of techniques that can be used to resolve stereoisomers of compounds from their racemic mixture can be found in Jean Jacques Andre Collet, Samuel H. Wilen, Enantiomers, Racemates and Resolutions, John Wiley & Sons, Inc. (1981).

4. INDICATIONS FOR USE OF HDAC INHIBITORS

[00149] HDAC is believed to contribute to the pathology and/or symptomology of several different diseases such that reduction of the activity of HDAC in a subject through inhibition may be used to therapeutically address these disease states. Examples of various diseases that may be treated using the HDAC inhibitors of the present invention are described herein. It is noted that additional diseases beyond those disclosed herein may be later identified as the biological roles that HDAC play in various pathways becomes more fully understood.

A. Undesirable or Uncontrolled Cell Proliferation

[00150] One set of indications that HDAC inhibitors of the present invention may be used to treat are those involving undesirable or uncontrolled cell proliferation. Such indications include benign tumors, various types of cancers such as primary tumors and tumor metastasis, restenosis (e.g. coronary, carotid, and cerebral lesions), abnormal stimulation of endothelial cells (atherosclerosis), insults to body tissue due to surgery, abnormal wound healing, abnormal angiogenesis, diseases that produce fibrosis of tissue, repetitive motion disorders, disorders of tissues that are not highly vascularized, and proliferative responses associated with organ transplants. More specific indications for HDAC inhibitors include, but are not limited to prostate cancer, lung cancer, acute leukemia, multiple myeloma, bladder carcinoma, renal carcinoma, breast carcinoma, colorectal carcinoma, neuroblastoma and melaoma.

[00151] In one embodiment, a method is provided for treating diseases associated with undesired and uncontrolled cell proliferation. The method comprises administering to a subject suffering from uncontrolled cell proliferation a therapeutically effective amount of a HDAC inhibitor according to the present invention, such that said uncontrolled cell proliferation is reduced. The particular dosage of the inhibitor to be used will depend on the severity of the

disease state, the route of administration, and related factors that can be determined by the attending physician. Generally, acceptable and effective daily doses are amounts sufficient to effectively slow or eliminate uncontrolled cell proliferation.

[00152] HDAC inhibitors according to the present invention may also be used in conjunction with other agents to inhibit undesirable and uncontrolled cell proliferation. Examples of other anti-cell proliferation agents that may be used in conjunction with the HDAC inhibitors of the present invention include, but are not limited to, retinoid acid and derivatives thereof, 2-methoxyestradiol, ANGIOSTATIN™ protein, ENDOSTATIN™ protein, suramin, squalamine, tissue inhibitor of metalloproteinase-1, tissue inhibitor of metalloproteinase-2, plasminogen activator inhibitor-1, plasminogen activator inhibitor-2, cartilage-derived inhibitor, paclitaxel, platelet factor 4, protamine sulphate (clupeine), sulphated chitin derivatives (prepared from queen crab shells), sulphated polysaccharide peptidoglycan complex (sp-pg), staurosporine, modulators of matrix metabolism, including for example, proline analogs ((l-azetidine-2-carboxylic acid (LACA), cishydroxyproline, d,l-3,4-dehydroproline, thiaproline], beta.-aminopropionitrile fumarate, 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; methotrexate, mitoxantrone, heparin, interferons, 2 macroglobulin-serum, chimp-3, chymostatin, beta.-cyclodextrin tetradecasulfate, eponemycin; fumagillin, gold sodium thiomalate, d-penicillamine (CDPT), beta.-1-anticollagenase-serum, alpha.2-antiplasmin, bisantrene, lobenzarit disodium, n-(2-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA", thalidomide; angostatic steroid, carbboxyaminolmidazole; metalloproteinase inhibitors such as BB94. Other anti-angiogenesis agents that may be used include antibodies, preferably monoclonal antibodies against these angiogenic growth factors: bFGF, aFGF, FGF-5, VEGF isoforms, VEGF-C, HGF/SF and Ang-1/Ang-2. Ferrara N. and Alitalo, K. "Clinical application of angiogenic growth factors and their inhibitors" (1999) Nature Medicine 5:1359-1364.

[00153] Generally, cells in benign tumors retain their differentiated features and do not divide in a completely uncontrolled manner. A benign tumor is usually localized and nonmetastatic. Specific types of benign tumors that can be treated using HDAC inhibitors of the present invention include hemangiomas, hepatocellular adenoma, cavernous haemangioma, focal nodular hyperplasia, acoustic neuromas, neurofibroma, bile duct adenoma, bile duct cystanoma,

fibroma, lipomas, leiomyomas, mesotheliomas, teratomas, myxomas, nodular regenerative hyperplasia, trachomas and pyogenic granulomas.

[00154] In the case of malignant tumors, cells become undifferentiated, do not respond to the body's growth control signals, and multiply in an uncontrolled manner. Malignant tumors are invasive and capable of spreading to distant sites (metastasizing). Malignant tumors are generally divided into two categories: primary and secondary. Primary tumors arise directly from the tissue in which they are found. Secondary tumors, or metastases, are tumors that originated elsewhere in the body but have now spread to distant organs. Common routes for metastasis are direct growth into adjacent structures, spread through the vascular or lymphatic systems, and tracking along tissue planes and body spaces (peritoneal fluid, cerebrospinal fluid, etc.)

[00155] Specific types of cancers or malignant tumors, either primary or secondary, that can be treated using the HDAC inhibitors of the present invention include, but are not limited to, leukemia, breast cancer, skin cancer, bone cancer, prostate cancer, liver cancer, lung cancer, brain cancer, cancer of the larynx, gallbladder, pancreas, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma of both ulcerating and papillary type, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, veticulum cell sarcoma, myeloma, giant cell tumor, small-cell lung tumor, gallstones, islet cell tumor, primary brain tumor, acute and chronic lymphocytic and granulocytic tumors, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, pheochromocytoma, mucosal neuronms, intestinal ganglloneuromas, hyperplastic corneal nerve tumor, marfanoid habitus tumor, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia and in situ carcinoma, neuroblastoma, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, mycosis fungoide, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic and other sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, glioblastoma multiforma, leukemias, lymphomas, malignant melanomas, epidermoid carcinomas, and other carcinomas and sarcomas.

[00156] The HDAC inhibitors of the present invention may also be used to treat abnormal cell proliferation due to insults to body tissue during surgery. These insults may arise as a result of a variety of surgical procedures such as joint surgery, bowel surgery, and cheloid scarring. Diseases that produce fibrotic tissue include emphysema. Repetitive motion disorders that may

be treated using the present invention include carpal tunnel syndrome. An example of a cell proliferative disorder that may be treated using the invention is a bone tumor.

[00157] Proliferative responses associated with organ transplantation that may be treated using HDAC inhibitors of the invention include proliferative responses contributing to potential organ rejections or associated complications. Specifically, these proliferative responses may occur during transplantation of the heart, lung, liver, kidney, and other body organs or organ systems.

[00158] Abnormal angiogenesis that may be treated using this invention include those abnormal angiogenesis accompanying rheumatoid arthritis, ischemic-reperfusion related brain edema and injury, cortical ischemia, ovarian hyperplasia and hypervascularity, (polycystic ovary syndrome), endometriosis, psoriasis, diabetic retinopathy, and other ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplastic), macular degeneration, corneal graft rejection, neurovascular glaucoma and Oster Webber syndrome.

[00159] Examples of diseases associated with uncontrolled angiogenesis that may be treated according to the present invention include, but are not limited to retinal/choroidal neovascularization and corneal neovascularization. Examples of retinal/choroidal neovascularization include, but are not limited to, Best's disease, myopia, optic pits, Stargardt's disease, Paget's disease, vein occlusion, artery occlusion, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum carotid abostructive diseases, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, diabetic retinopathy, macular degeneration,, Bechets disease, infections causing a retinitis or chroiditis, presumed ocular histoplasmosis, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications, diseases associated with rubesis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy. Examples of corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogrens, acne rosacea, phlyctenulosis, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, polyarteritis, Wegener sarcoidosis, Scleritis,

periphigoid radial keratotomy, neovascular glaucoma and retrothalic fibroplasia, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections and Kaposi sarcoma.

[00160] Chronic inflammatory diseases associated with uncontrolled angiogenesis may also be treated using HDAC inhibitors of the present invention. Chronic inflammation depends on continuous formation of capillary sprouts to maintain an influx of inflammatory cells. The influx and presence of the inflammatory cells produce granulomas and thus maintains the chronic inflammatory state. Inhibition of angiogenesis using a HDAC inhibitor alone or in conjunction with other anti-inflammatory agents may prevent the formation of the granulosmas and thus alleviate the disease. Examples of chronic inflammatory diseases include, but are not limited to, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis, and rheumatoid arthritis.

[00161] Inflammatory bowel diseases such as Crohn's disease and ulcerative colitis are characterized by chronic inflammation and angiogenesis at various sites in the gastrointestinal tract. For example, Crohn's disease occurs as a chronic transmural inflammatory disease that most commonly affects the distal ileum and colon but may also occur in any part of the gastrointestinal tract from the mouth to the anus and perianal area. Patients with Crohn's disease generally have chronic diarrhea associated with abdominal pain, fever, anorexia, weight loss and abdominal swelling. Ulcerative colitis is also a chronic, nonspecific, inflammatory and ulcerative disease arising in the colonic mucosa and is characterized by the presence of bloody diarrhea. These inflammatory bowel diseases are generally caused by chronic granulomatous inflammation throughout the gastrointestinal tract, involving new capillary sprouts surrounded by a cylinder of inflammatory cells. Inhibition of angiogenesis by these inhibitors should inhibit the formation of the sprouts and prevent the formation of granulomas. Inflammatory bowel diseases also exhibit extra intestinal manifestations, such as skin lesions. Such lesions are characterized by inflammation and angiogenesis and can occur at many sites other the gastrointestinal tract. Inhibition of angiogenesis by HDAC inhibitors according to the present invention can reduce the influx of inflammatory cells and prevent lesion formation.

[00162] Sarcoidosis, another chronic inflammatory disease, is characterized as a multisystem granulomatous disorder. The granulomas of this disease can form anywhere in the

body. Thus, the symptoms depend on the site of the granulomas and whether the disease is active. The granulomas are created by the angiogenic capillary sprouts providing a constant supply of inflammatory cells. By using HDAC inhibitors according to the present invention to inhibit angiogenesis, such granulomas formation can be inhibited. Psoriasis, also a chronic and recurrent inflammatory disease, is characterized by papules and plaques of various sizes. Treatment using these inhibitors alone or in conjunction with other anti-inflammatory agents should prevent the formation of new blood vessels necessary to maintain the characteristic lesions and provide the patient relief from the symptoms.

[00163] Rheumatoid arthritis (RA) is also a chronic inflammatory disease characterized by non-specific inflammation of the peripheral joints. It is believed that the blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis. Treatment using HDAC inhibitors according to the present invention alone or in conjunction with other anti-RA agents may prevent the formation of new blood vessels necessary to maintain the chronic inflammation and provide the RA patient relief from the symptoms.

5. COMPOSITIONS COMPRISING HDAC INHIBITORS

[00164] A wide variety of compositions and administration methods may be used in conjunction with the HDAC inhibitors of the present invention. Such compositions may include, in addition to the HDAC inhibitors of the present invention, conventional pharmaceutical excipients, and other conventional, pharmaceutically inactive agents. Additionally, the compositions may include active agents in addition to the HDAC inhibitors of the present invention. These additional active agents may include additional compounds according to the invention, or one or more other pharmaceutically active agents.

[00165] The compositions may be in gaseous, liquid, semi-liquid or solid form, formulated in a manner suitable for the route of administration to be used. For oral administration, capsules and tablets are typically used. For parenteral administration, reconstitution of a lyophilized powder, prepared as described herein, is typically used.

[00166] Compositions comprising HDAC inhibitors of the present invention may be administered or coadministered orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local delivery (for example by catheter or stent), subcutaneously, intraadiposally, intraarticularly, or intrathecally. The compounds and/or compositions according to the invention may also be administered or coadministered in slow release dosage forms.

[00167] The HDAC inhibitors and compositions comprising them may be administered or coadministered in any conventional dosage form. Coadministration in the context of this invention is intended to mean the administration of more than one therapeutic agents, one of which includes a HDAC inhibitor, in the course of a coordinated treatment to achieve an improved clinical outcome. Such coadministration may also be coextensive, that is, occurring during overlapping periods of time.

[00168] Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application may optionally include one or more of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; agents for the adjustment of tonicity such as sodium chloride or dextrose, and agents for adjusting the acidity or alkalinity of the composition, such as alkaline or acidifying agents or buffers like carbonates, bicarbonates, phosphates, hydrochloric acid, and organic acids like acetic and citric acid. Parenteral preparations may optionally be enclosed in ampules, disposable syringes or single or multiple dose vials made of glass, plastic or other suitable material.

[00169] When HDAC inhibitors according to the present invention exhibit insufficient solubility, methods for solubilizing the compounds may be used. Such methods are known to those of skill in this art, and include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as TWEEN, or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as prodrugs of the compounds may also be used in formulating effective pharmaceutical compositions.

[00170] Upon mixing or adding HDAC inhibitors according to the present invention to a composition, a solution, suspension, emulsion or the like may be formed. The form of the resulting composition will depend upon a number of factors, including the intended mode of administration, and the solubility of the compound in the selected carrier or vehicle. The effective concentration needed to ameliorate the disease being treated may be empirically determined.

[00171] Compositions according to the present invention are optionally provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, dry powders for inhalers, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the compounds, particularly the pharmaceutically acceptable salts, preferably the sodium salts, thereof. The pharmaceutically therapeutically active compounds and derivatives thereof are typically formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms, as used herein, refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include ampoules and syringes individually packaged tablet or capsule. Unit-dose forms may be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pint or gallons. Hence, multiple dose form is a multiple of unit-doses that are not segregated in packaging.

[00172] In addition to one or more HDAC inhibitors according to the present invention, the composition may comprise: a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acaciagelatin, glucose, molasses, polyvinylpyrrolidine, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound

as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of auxiliary substances such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents. Actual methods of preparing such dosage forms are known in the art, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition, 1975. The composition or formulation to be administered will, in any event, contain a sufficient quantity of a HDAC inhibitor of the present invention to reduce HDAC activity *in vivo*, thereby treating the disease state of the subject.

[00173] Dosage forms or compositions may optionally comprise one or more HDAC inhibitors according to the present invention in the range of 0.005% to 100% (weight/weight) with the balance comprising additional substances such as those described herein. For oral administration, a pharmaceutically acceptable composition may optionally comprise any one or more commonly employed excipients, such as, for example pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, talcum, cellulose derivatives, sodium crosscarmellose, glucose, sucrose, magnesium carbonate, sodium saccharin, talcum. Such compositions include solutions, suspensions, tablets, capsules, powders, dry powders for inhalers and sustained release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as collagen, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others. Methods for preparing these formulations are known to those skilled in the art. The compositions may optionally contain 0.01%-100% (weight/weight) of one or more HDAC inhibitors, optionally 0.1-95%, and optionally 1-95%.

[00174] Salts, preferably sodium salts, of the HDAC inhibitors may be prepared with carriers that protect the compound against rapid elimination from the body, such as time release formulations or coatings. The formulations may further include other active compounds to obtain desired combinations of properties.

A. Formulations For Oral Administration

[00175] Oral pharmaceutical dosage forms may be as a solid, gel or liquid. Examples of solid dosage forms include, but are not limited to tablets, capsules, granules, and bulk powders. More specific examples of oral tablets include compressed, chewable lozenges and tablets that may be enteric-coated, sugar-coated or film-coated. Examples of capsules include hard or soft gelatin capsules. Granules and powders may be provided in non-effervescent or effervescent forms. Each may be combined with other ingredients known to those skilled in the art.

[00176] In certain embodiments, HDAC inhibitors according to the present invention are provided as solid dosage forms, preferably capsules or tablets. The tablets, pills, capsules, troches and the like may optionally contain one or more of the following ingredients, or compounds of a similar nature: a binder; a diluent; a disintegrating agent; a lubricant; a glidant; a sweetening agent; and a flavoring agent.

[00177] Examples of binders that may be used include, but are not limited to, microcrystalline cellulose, gum tragacanth, glucose solution, acacia mucilage, gelatin solution, sucrose and starch paste.

[00178] Examples of lubricants that may be used include, but are not limited to, talc, starch, magnesium or calcium stearate, lycopodium and stearic acid.

[00179] Examples of diluents that may be used include, but are not limited to, lactose, sucrose, starch, kaolin, salt, mannitol and dicalcium phosphate.

[00180] Examples of glidants that may be used include, but are not limited to, colloidal silicon dioxide.

[00181] Examples of disintegrating agents that may be used include, but are not limited to, crosscarmellose sodium, sodium starch glycolate, alginic acid, corn starch, potato starch, bentonite, methylcellulose, agar and carboxymethylcellulose.

[00182] Examples of coloring agents that may be used include, but are not limited to, any of the approved certified water soluble FD and C dyes, mixtures thereof; and water insoluble FD and C dyes suspended on alumina hydrate.

[00183] Examples of sweetening agents that may be used include, but are not limited to, sucrose, lactose, mannitol and artificial sweetening agents such as sodium cyclamate and saccharin, and any number of spray dried flavors.

[00184] Examples of flavoring agents that may be used include, but are not limited to, natural flavors extracted from plants such as fruits and synthetic blends of compounds that produce a pleasant sensation, such as, but not limited to peppermint and methyl salicylate.

[00185] Examples of wetting agents that may be used include, but are not limited to, propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene laural ether.

[00186] Examples of emetic-coatings that may be used include, but are not limited to, fatty acids, fats, waxes, shellac, ammoniated shellac and cellulose acetate phthalates.

[00187] Examples of film coatings that may be used include, but are not limited to, hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000 and cellulose acetate phthalate.

[00188] If oral administration is desired, the salt of the compound may optionally be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

[00189] When the dosage unit form is a capsule, it may optionally additionally comprise a liquid carrier such as a fatty oil. In addition, dosage unit forms may optionally additionally comprise various other materials that modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents.

[00190] Compounds according to the present invention may also be administered as a component of an elixir, suspension, syrup, wafer, sprinkle, chewing gum or the like. A syrup may optionally comprise, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

[00191] The HDAC inhibitors of the present invention may also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antacids, H₂ blockers, and diuretics. For example, if a compound is used

for treating asthma or hypertension, it may be used with other bronchodilators and antihypertensive agents, respectively.

[00192] Examples of pharmaceutically acceptable carriers that may be included in tablets comprising HDAC inhibitors of the present invention include, but are not limited to binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, and wetting agents. Enteric-coated tablets, because of the enteric-coating, resist the action of stomach acid and dissolve or disintegrate in the neutral or alkaline intestines. Sugar-coated tablets may be compressed tablets to which different layers of pharmaceutically acceptable substances are applied. Film-coated tablets may be compressed tablets that have been coated with polymers or other suitable coating. Multiple compressed tablets may be compressed tablets made by more than one compression cycle utilizing the pharmaceutically acceptable substances previously mentioned. Coloring agents may also be used in tablets. Flavoring and sweetening agents may be used in tablets, and are especially useful in the formation of chewable tablets and lozenges.

[00193] Examples of liquid oral dosage forms that may be used include, but are not limited to, aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules.

[00194] Examples of aqueous solutions that may be used include, but are not limited to, elixirs and syrups. As used herein, elixirs refer to clear, sweetened, hydroalcoholic preparations. Examples of pharmaceutically acceptable carriers that may be used in elixirs include, but are not limited to solvents. Particular examples of solvents that may be used include glycerin, sorbitol, ethyl alcohol and syrup. As used herein, syrups refer to concentrated aqueous solutions of a sugar, for example, sucrose. Syrups may optionally further comprise a preservative.

[00195] Emulsions refer to two-phase systems in which one liquid is dispersed in the form of small globules throughout another liquid. Emulsions may optionally be oil-in-water or water-in-oil emulsions. Examples of pharmaceutically acceptable carriers that may be used in emulsions include, but are not limited to non-aqueous liquids, emulsifying agents and preservatives.

[00196] Examples of pharmaceutically acceptable substances that may be used in non-effervescent granules, to be reconstituted into a liquid oral dosage form, include diluents, sweeteners and wetting agents.

[00197] Examples of pharmaceutically acceptable substances that may be used in effervescent granules, to be reconstituted into a liquid oral dosage form, include organic acids and a source of carbon dioxide.

[00198] Coloring and flavoring agents may optionally be used in all of the above dosage forms.

[00199] Particular examples of preservatives that may be used include glycerin, methyl and propylparaben, benzoic acid, sodium benzoate and alcohol.

[00200] Particular examples of non-aqueous liquids that may be used in emulsions include mineral oil and cottonseed oil.

[00201] Particular examples of emulsifying agents that may be used include gelatin, acacia, tragacanth, bentonite, and surfactants such as polyoxyethylene sorbitan monooleate.

[00202] Particular examples of suspending agents that may be used include sodium carboxymethylcellulose, pectin, tragacanth, Veegum and acacia. Diluents include lactose and sucrose. Sweetening agents include sucrose, syrups, glycerin and artificial sweetening agents such as sodium cyclamate and saccharin.

[00203] Particular examples of wetting agents that may be used include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene lauryl ether.

[00204] Particular examples of organic acids that may be used include citric and tartaric acid.

[00205] Sources of carbon dioxide that may be used in effervescent compositions include sodium bicarbonate and sodium carbonate. Coloring agents include any of the approved certified water soluble FD and C dyes, and mixtures thereof.

[00206] Particular examples of flavoring agents that may be used include natural flavors extracted from plants such fruits, and synthetic blends of compounds that produce a pleasant taste sensation.

[00207] For a solid dosage form, the solution or suspension, in for example propylene carbonate, vegetable oils or triglycerides, is preferably encapsulated in a gelatin capsule. Such solutions, and the preparation and encapsulation thereof, are disclosed in U.S. Pat. Nos. 4,328,245; 4,409,239; and 4,410,545. For a liquid dosage form, the solution, eg., for example, in a polyethylene glycol, may be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, e.g. water, to be easily measured for administration.

[00208] Alternatively, liquid or semi-solid oral formulations may be prepared by dissolving or dispersing the active compound or salt in vegetable oils, glycols, triglycerides, propylene glycol esters (e.g. propylene carbonate) and other such carriers, and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells. Other useful formulations include those set forth in U.S. Pat. Nos. Re 28,819 and 4,358,603.

B. Injectables, Solutions and Emulsions

[00209] The present invention is also directed to compositions designed to administer the HDAC inhibitors of the present invention by parenteral administration, generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables may be prepared in any conventional form, for example as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

[00210] Examples of excipients that may be used in conjunction with injectables according to the present invention include, but are not limited to water, saline, dextrose, glycerol or ethanol. The injectable compositions may also optionally comprise minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins. Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e.g., U.S. Pat. No. 3,710,795) is also contemplated herein. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject.

[00211] Parenteral administration of the formulations includes intravenous, subcutaneous and intramuscular administrations. Preparations for parenteral administration include sterile

solutions ready for injection, sterile dry soluble products, such as the lyophilized powders described herein, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

[00212] When administered intravenously, examples of suitable carriers include, but are not limited to physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

[00213] Example of pharmaceutically acceptable carriers that may optionally be used in parenteral preparations include, but are not limited to aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances.

[00214] Examples of aqueous vehicles that may optionally be used include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection.

[00215] Examples of nonaqueous parenteral vehicles that may optionally be used include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil.

[00216] Antimicrobial agents in bacteriostatic or fungistatic concentrations may be added to parenteral preparations, particularly when the preparations are packaged in multiple-dose containers and thus designed to be stored and multiple aliquots to be removed. Examples of antimicrobial agents that may be used include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride.

[00217] Examples of isotonic agents that may be used include sodium chloride and dextrose. Examples of buffers that may be used include phosphate and citrate. Examples of antioxidants that may be used include sodium bisulfate. Examples of local anesthetics that may be used include procaine hydrochloride. Examples of suspending and dispersing agents that may be used include sodium carboxymethylcellulose, hydroxypropyl methylcellulose and

polyvinylpyrrolidone. Examples of emulsifying agents that may be used include Polysorbate 80 (Tween 80). A sequestering or chelating agent of metal ions include EDTA.

[00218] Pharmaceutical carriers may also optionally include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

[00219] The concentration of a HDAC inhibitor in the parenteral formulation may be adjusted so that an injection administers a pharmaceutically effective amount sufficient to produce the desired pharmacological effect. The exact concentration of a HDAC inhibitor and/or dosage to be used will ultimately depend on the age, weight and condition of the patient or animal as is known in the art.

[00220] Unit-dose parenteral preparations may be packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile, as is known and practiced in the art.

[00221] Injectables may be designed for local and systemic administration. Typically a therapeutically effective dosage is formulated to contain a concentration of at least about 0.1% w/w up to about 90% w/w or more, preferably more than 1% w/w of the HDAC inhibitor to the treated tissue(s). The HDAC inhibitor may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment will be a function of the location of where the composition is parenterally administered, the carrier and other variables that may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data. It is to be noted that concentrations and dosage values may also vary with the age of the individual treated. It is to be further understood that for any particular subject, specific dosage regimens may need to be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations. Hence, the concentration ranges set forth herein are intended to be exemplary and are not intended to limit the scope or practice of the claimed formulations.

[00222] The HDAC inhibitor may optionally be suspended in micronized or other suitable form or may be derivatized to produce a more soluble active product or to produce a prodrug. The form of the resulting mixture depends upon a number of factors, including the intended

mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the disease state and may be empirically determined.

C. Lyophilized Powders

[00223] The HDAC inhibitors of the present invention may also be prepared as lyophilized powders, which can be reconstituted for administration as solutions, emulsions and other mixtures. The lyophilized powders may also be formulated as solids or gels.

[00224] Sterile, lyophilized powder may be prepared by dissolving the sodium salt in a sodium phosphate buffer solution containing dextrose or other suitable excipient. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. Briefly, the lyophilized powder may optionally be prepared by dissolving dextrose, sorbitol, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent, about 1-20%, preferably about 5 to 15%, in a suitable buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, typically, about neutral pH. Then, a HDAC inhibitor is added to the resulting mixture, preferably above room temperature, more preferably at about 30-35 °C, and stirred until it dissolves. The resulting mixture is diluted by adding more buffer to a desired concentration. The resulting mixture is sterile filtered or treated to remove particulates and to insure sterility, and apportioned into vials for lyophilization. Each vial may contain a single dosage or multiple dosages of the HDAC inhibitor.

D. Topical Administration

[00225] The HDAC inhibitors of the present invention may also be administered as topical mixtures. Topical mixtures may be used for local and systemic administration. The resulting mixture may be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

[00226] The HDAC inhibitors may be formulated as aerosols for topical application, such as by inhalation (see, U.S. Pat. Nos. 4,044,126, 4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will typically diameters of less than 50 microns, preferably less than 10 microns.

[00227] The HDAC inhibitors may also be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the HDAC inhibitor alone or in combination with other pharmaceutically acceptable excipients can also be administered.

E. Formulations For Other Routes of Administration

[00228] Depending upon the disease state being treated, other routes of administration, such as topical application, transdermal patches, a rectal administration, may also be used. For example, pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories are used herein mean solid bodies for insertion into the rectum that melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax, (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. The typical weight of a rectal suppository is about 2 to 3 gm. Tablets and capsules for rectal administration may be manufactured using the same

pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

F. Examples of Formulations

[00229] The following are particular examples of oral, intravenous and tablet formulations that may optionally be used with compounds of the present invention. It is noted that these formulations may be varied depending on the particular compound being used and the indication for which the formulation is going to be used.

ORAL FORMULATION

Compound of the Present Invention	10-100 mg
Citric Acid Monohydrate	105 mg
Sodium Hydroxide	18 mg
Flavoring	
Water	q.s. to 100 mL

INTRAVENOUS FORMULATION

Compound of the Present Invention	0.1-10 mg
Dextrose Monohydrate	q.s. to make isotonic
Citric Acid Monohydrate	1.05 mg
Sodium Hydroxide	0.18 mg
Water for Injection	q.s. to 1.0 mL

TABLET FORMULATION

Compound of the Present Invention	1%
Microcrystalline Cellulose	73%
Stearic Acid	25%
Colloidal Silica	1%.

6. KITS COMPRISING HDAC INHIBITORS

[00230] The invention is also directed to kits and other articles of manufacture for treating diseases associated with HDAC. It is noted that diseases are intended to cover all conditions for which the HDAC possesses activity that contributes to the pathology and/or symptomology of the condition.

[00231] In one embodiment, a kit is provided that comprises a composition comprising at least one HDAC inhibitor of the present invention in combination with instructions. The instructions may indicate the disease state for which the composition is to be administered, storage information, dosing information and/or instructions regarding how to administer the composition. The kit may also comprise packaging materials. The packaging material may comprise a container for housing the composition. The kit may also optionally comprise additional components, such as syringes for administration of the composition. The kit may comprise the composition in single or multiple dose forms.

[00232] In another embodiment, an article of manufacture is provided that comprises a composition comprising at least one HDAC inhibitor of the present invention in combination with packaging materials. The packaging material may comprise a container for housing the composition. The container may optionally comprise a label indicating the disease state for which the composition is to be administered, storage information, dosing information and/or instructions regarding how to administer the composition. The kit may also optionally comprise additional components, such as syringes for administration of the composition. The kit may comprise the composition in single or multiple dose forms.

[00233] It is noted that the packaging material used in kits and articles of manufacture according to the present invention may form a plurality of divided containers such as a divided bottle or a divided foil packet. The container can be in any conventional shape or form as known in the art which is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container that is employed will depend on the exact dosage form involved, for example a conventional cardboard box would not generally be used to hold a liquid suspension. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle that is in turn contained within a box. Typically the kit includes directions for the administration of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (e.g., oral,

topical, transdermal and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician.

[00234] One particular example of a kit according to the present invention is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process recesses are formed in the plastic foil. The recesses have the size and shape of individual tablets or capsules to be packed or may have the size and shape to accommodate multiple tablets and/or capsules to be packed. Next, the tablets or capsules are placed in the recesses accordingly and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are individually sealed or collectively sealed, as desired, in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

[00235] Another specific embodiment of a kit is a dispenser designed to dispense the daily doses one at a time in the order of their intended use. Preferably, the dispenser is equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter that indicates the number of daily doses that has been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

7. COMBINATION THERAPY

[00236] A wide variety therapeutic agents may have a therapeutic additive or synergistic effect with HDAC inhibitors according to the present invention. Such therapeutic agents may additively or synergistically combine with the HDAC inhibitors to inhibit undesirable cell

growth, such as inappropriate cell growth resulting in undesirable benign conditions or tumor growth.

[00237] In one embodiment, a method is provided for treating a cell proliferative disease state comprising treating cells with a compound according to the present invention in combination with an anti-proliferative agent, wherein the cells are treated with the compound according to the present invention before, at the same time, and/or after the cells are treated with the anti-proliferative agent, referred to herein as combination therapy. It is noted that treatment of one agent before another is referred to herein as sequential therapy, even if the agents are also administered together. It is noted that combination therapy is intended to cover when agents are administered before or after each other (sequential therapy) as well as when the agents are administered at the same time.

[00238] Examples of therapeutic agents that may be used in combination with HDAC inhibitors include, but are not limited to, alkylating agents, antibiotic agents, antimetabolic agents, hormonal agents, plant-derived agents, and biologic agents.

[00239] Alkylating agents are polyfunctional compounds that have the ability to substitute alkyl groups for hydrogen ions. Examples of alkylating agents include, but are not limited to, bis(chloroethyl)amines (nitrogen mustards, e.g. chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan, uracil mustard), aziridines (e.g. thiotepa), alkyl alkone sulfonates (e.g. busulfan), nitrosoureas (e.g. carmustine, lomustine, streptozocin), nonclassic alkylating agents (altretamine, dacarbazine, and procarbazine), platinum compounds (carboplatin and cisplatin). These compounds react with phosphate, amino, hydroxyl, sulphydryl, carboxyl, and imidazole groups. Under physiological conditions, these drugs ionize and produce positively charged ion that attach to susceptible nucleic acids and proteins, leading to cell cycle arrest and/or cell death. Combination therapy including a HDAC inhibitor and an alkylating agent may have therapeutic synergistic effects on cancer and reduce side effects associated with these chemotherapeutic agents.

[00240] Antibiotic agents are a group of drugs that produced in a manner similar to antibiotics as a modification of natural products. Examples of antibiotic agents include, but are not limited to, anthracyclines (e.g. doxorubicin, daunorubicin, epirubicin, idarubicin and anthracenedione), mitomycin C, bleomycin, dactinomycin, plicamycin. These antibiotic agents

interferes with cell growth by targeting different cellular components. For example, anthracyclines are generally believed to interfere with the action of DNA topoisomerase II in the regions of transcriptionally active DNA, which leads to DNA strand scissions. Bleomycin is generally believed to chelate iron and forms an activated complex, which then binds to bases of DNA, causing strand scissions and cell death. Combination therapy including a HDAC INHIBITOR and an antibiotic agent may have therapeutic synergistic effects on cancer and reduce side affects associated with these chemotherapeutic agents.

[00241] Antimetabolic agents are a group of drugs that interfere with metabolic processes vital to the physiology and proliferation of cancer cells. Actively proliferating cancer cells require continuous synthesis of large quantities of nucleic acids, proteins, lipids, and other vital cellular constituents. Many of the antimetabolites inhibit the synthesis of purine or pyrimidine nucleosides or inhibit the enzymes of DNA replication. Some antimetabolites also interfere with the synthesis of ribonucleosides and RNA and/or amino acid metabolism and protein synthesis as well. By interfering with the synthesis of vital cellular constituents, antimetabolites can delay or arrest the growth of cancer cells. Examples of antimetabolic agents include, but are not limited to, fluorouracil (5-FU), floxuridine (5-FUDR), methotrexate, leucovorin, hydroxyurea, thioguanine (6-TG), mercaptopurine (6-MP), cytarabine, pentostatin, fludarabine phosphate, cladribine (2-CDA), asparaginase, and gemcitabine. Combination therapy including a HDAC INHIBITOR and a antimetabolic agent may have therapeutic synergistic effects on cancer and reduce side affects associated with these chemotherapeutic agents.

[00242] Hormonal agents are a group of drug that regulate the growth and development of their target organs. Most of the hormonal agents are sex steroids and their derivatives and analogs thereof, such as estrogens, androgens, and progestins. These hormonal agents may serve as antagonists of receptors for the sex steroids to down regulate receptor expression and transcription of vital genes. Examples of such hormonal agents are synthetic estrogens (e.g. diethylstibestrol), antiestrogens (e.g. tamoxifen, toremifene, fluoxymesterol and raloxifene), antiandrogens (bicalutamide, nilutamide, flutamide), aromatase inhibitors (e.g., aminoglutethimide, anastrozole and tetrazole), ketoconazole, goserelin acetate, leuprolide, megestrol acetate and mifepristone. Combination therapy including a HDAC INHIBITOR and a

hormonal agent may have therapeutic synergistic effects on cancer and reduce sides affects associated with these chemotherapeutic agents.

[00243] Plant-derived agents are a group of drugs that are derived from plants or modified based on the molecular structure of the agents. Examples of plant-derived agents include, but are not limited to, vinca alkaloids (e.g., vincristine, vinblastine, vindesine, vinzolidine and vinorelbine), podophyllotoxins (e.g., etoposide (VP-16) and teniposide (VM-26)), taxanes (e.g., paclitaxel and docetaxel). These plant-derived agents generally act as antimitotic agents that bind to tubulin and inhibit mitosis. Podophyllotoxins such as etoposide are believed to interfere with DNA synthesis by interacting with topoisomerase II, leading to DNA strand scission. Combination therapy including a HDAC INHIBITOR and a plant-derived agent may have therapeutic synergistic effects on cancer and reduce sides affects associated with these chemotherapeutic agents.

[00244] Biologic agents are a group of biomolecules that elicit cancer/tumor regression when used alone or in combination with chemotherapy and/or radiotherapy. Examples of biologic agents include, but are not limited to, immuno-modulating proteins such as cytokines, monoclonal antibodies against tumor antigens, tumor suppressor genes, and cancer vaccines. Combination therapy including a HDAC INHIBITOR and a biologic agent may have therapeutic synergistic effects on cancer, enhance the patient's immune responses to tumorigenic signals, and reduce potential sides affects associated with this chemotherapeutic agent.

[00245] Cytokines possess profound immunomodulatory activity. Some cytokines such as interleukin-2 (IL-2, aldesleukin) and interferon have demonstrated antitumor activity and have been approved for the treatment of patients with metastatic renal cell carcinoma and metastatic malignant melanoma. IL-2 is a T-cell growth factor that is central to T-cell-mediated immune responses. The selective antitumor effects of IL-2 on some patients are believed to be the result of a cell-mediated immune response that discriminate between self and nonself. Examples of interleukins that may be used in conjunction with HDAC INHIBITOR include, but are not limited to, interleukin 2 (IL-2), and interleukin 4 (IL-4), interleukin 12 (IL-12).

[00246] Interferon include more than 23 related subtypes with overlapping activities, all of the IFN subtypes within the scope of the present invention. IFN has demonstrated activity against many solid and hematologic malignancies, the later appearing to be particularly sensitive.

[00247] Other cytokines that may be used in conjunction with a HDAC INHIBITOR include those cytokines that exert profound effects on hematopoiesis and immune functions. Examples of such cytokines include, but are not limited to erythropoietin, granulocyte-CSF (filgrastim), and granulocyte, macrophage-CSF (sargramostim). These cytokines may be used in conjunction with a HDAC INHIBITOR to reduce chemotherapy-induced myelopoietic toxicity.

[00248] Other immuno-modulating agents other than cytokines may also be used in conjunction with a HDAC INHIBITOR to inhibit abnormal cell growth. Examples of such immuno-modulating agents include, but are not limited to bacillus Calmette-Guerin, levamisole, and octreotide, a long-acting octapeptide that mimics the effects of the naturally occurring hormone somatostatin.

[00249] Monoclonal antibodies against tumor antigens are antibodies elicited against antigens expressed by tumors, preferably tumor-specific antigens. For example, monoclonal antibody HERCEPTIN® (Trastuzumab) is raised against human epidermal growth factor receptor2 (HER2) that is overexpressed in some breast tumors including metastatic breast cancer. Overexpression of HER2 protein is associated with more aggressive disease and poorer prognosis in the clinic. HERCEPTIN® is used as a single agent for the treatment of patients with metastatic breast cancer whose tumors over express the HER2 protein. Combination therapy including HDAC INHIBITOR and HERCEPTIN® may have therapeutic synergistic effects on tumors, especially on metastatic cancers.

[00250] Another example of monoclonal antibodies against tumor antigens is RITUXAN® (Rituximab) that is raised against CD20 on lymphoma cells and selectively deplete normal and malignant CD20⁺ pre-B and mature B cells. RITUXAN® is used as single agent for the treatment of patients with relapsed or refractory low-grade or follicular, CD20+, B cell non-Hodgkin's lymphoma. Combination therapy including HDAC INHIBITOR and RITUXAN® may have therapeutic synergistic effects not only on lymphoma, but also on other forms or types of malignant tumors.

[00251] Tumor suppressor genes are genes that function to inhibit the cell growth and division cycles, thus preventing the development of neoplasia. Mutations in tumor suppressor genes cause the cell to ignore one or more of the components of the network of inhibitory signals, overcoming the cell cycle check points and resulting in a higher rate of controlled cell growth—

cancer. Examples of the tumor suppressor genes include, but are not limited to, *DPC-4*, *NF-1*, *NF-2*, *RB*, *p53*, *WT1*, *BRCA1* and *BRCA2*.

[00252] *DPC-4* is involved in pancreatic cancer and participates in a cytoplasmic pathway that inhibits cell division. *NF-1* codes for a protein that inhibits Ras, a cytoplasmic inhibitory protein. *NF-1* is involved in neurofibroma and pheochromocytomas of the nervous system and myeloid leukemia. *NF-2* encodes a nuclear protein that is involved in meningioma, schwannoma, and ependymoma of the nervous system. *RB* codes for the pRB protein, a nuclear protein that is a major inhibitor of cell cycle. *RB* is involved in retinoblastoma as well as bone, bladder, small cell lung and breast cancer. *P53* codes for p53 protein that regulates cell division and can induce apoptosis. Mutation and/or inaction of p53 is found in a wide ranges of cancers. *WT1* is involved in Wilms tumor of the kidneys. *BRCA1* is involved in breast and ovarian cancer, and *BRCA2* is involved in breast cancer. The tumor suppressor gene can be transferred into the tumor cells where it exerts its tumor suppressing functions. Combination therapy including a HDAC INHIBITOR and a tumor suppressor may have therapeutic synergistic effects on patients suffering from various forms of cancers.

[00253] Cancer vaccines are a group of agents that induce the body's specific immune response to tumors. Most of cancer vaccines under research and development and clinical trials are tumor-associated antigens (TAAs). TAA are structures (i.e. proteins, enzymes or carbohydrates) which are present on tumor cells and relatively absent or diminished on normal cells. By virtue of being fairly unique to the tumor cell, TAAs provide targets for the immune system to recognize and cause their destruction. Examples of TAAs include, but are not limited to gangliosides (GM2), prostate specific antigen (PSA), α -fetoprotein (AFP), carcinoembryonic antigen (CEA) (produced by colon cancers and other adenocarcinomas, e.g. breast, lung, gastric, and pancreas cancers), melanoma associated antigens (MART-1, gp100, MAGE 1,3 tyrosinase), papillomavirus E6 and E7 fragments, whole cells or portions/lysates of analogous tumor cells and allogeneic tumor cells.

[00254] An adjuvant may be used to augment the immune response to TAAs. Examples of adjuvants include, but are not limited to, bacillus Calmette-Guerin (BCG), endotoxin lipopolysaccharides, keyhole limpet hemocyanin (GKLH), interleukin-2 (IL-2), granulocyte-

macrophage colony-stimulating factor (GM-CSF) and cytoxin, a chemotherapeutic agent which is believed to reduce tumor-induced suppression when given in low doses.

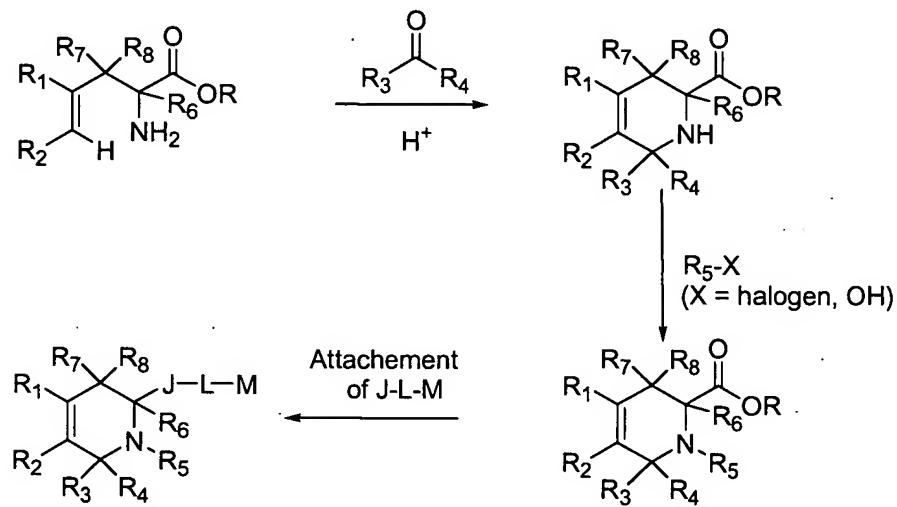
EXAMPLES

1. Synthetic Schemes For HDAC Inhibitors

[00255] HDAC inhibitors according to the present invention may be synthesized according to a variety of reaction schemes. Some illustrative schemes are provided herein in the examples. Other reaction schemes could be readily devised by those skilled in the art.

[00256] Scheme 1 (below) illustrates how a six membered ring may present in the compounds of the present invention may be formed via a Pictet Spengler reaction. As illustrated, an aldehyde or ketone $[C(O)R_3R_4]$ is reacted with an olefin comprising an amine gamma to the olefin.

Scheme 1

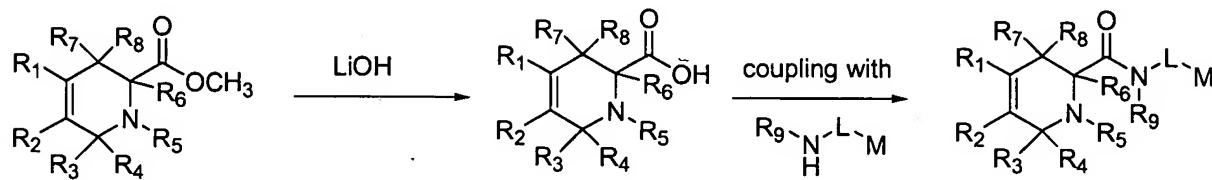


[00257] As illustrated in Scheme 1, the leader group (J, L) and metal complexing substituent M may be added after the Pictet Spengler reaction through a reaction with the ester moiety shown.

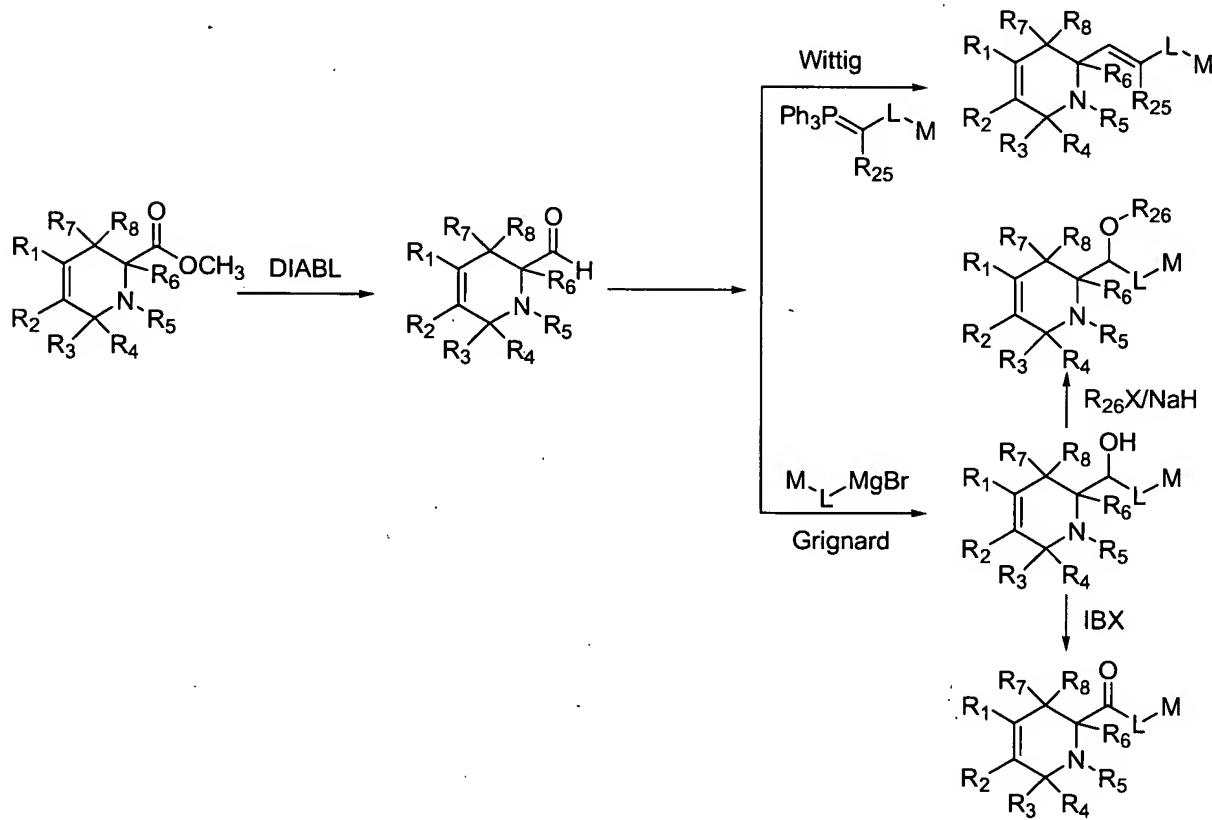
[00258] Schemes 2, 3, 4 and 5 (below) illustrate a variety of different coupling reactions that may be used to add the leader group (J, L) and metal complexing substituent M through

elaboration of the ester moiety shown. It should be recognized that further reaction schemes may be designed, as would be understood by one of ordinary skill in the art.

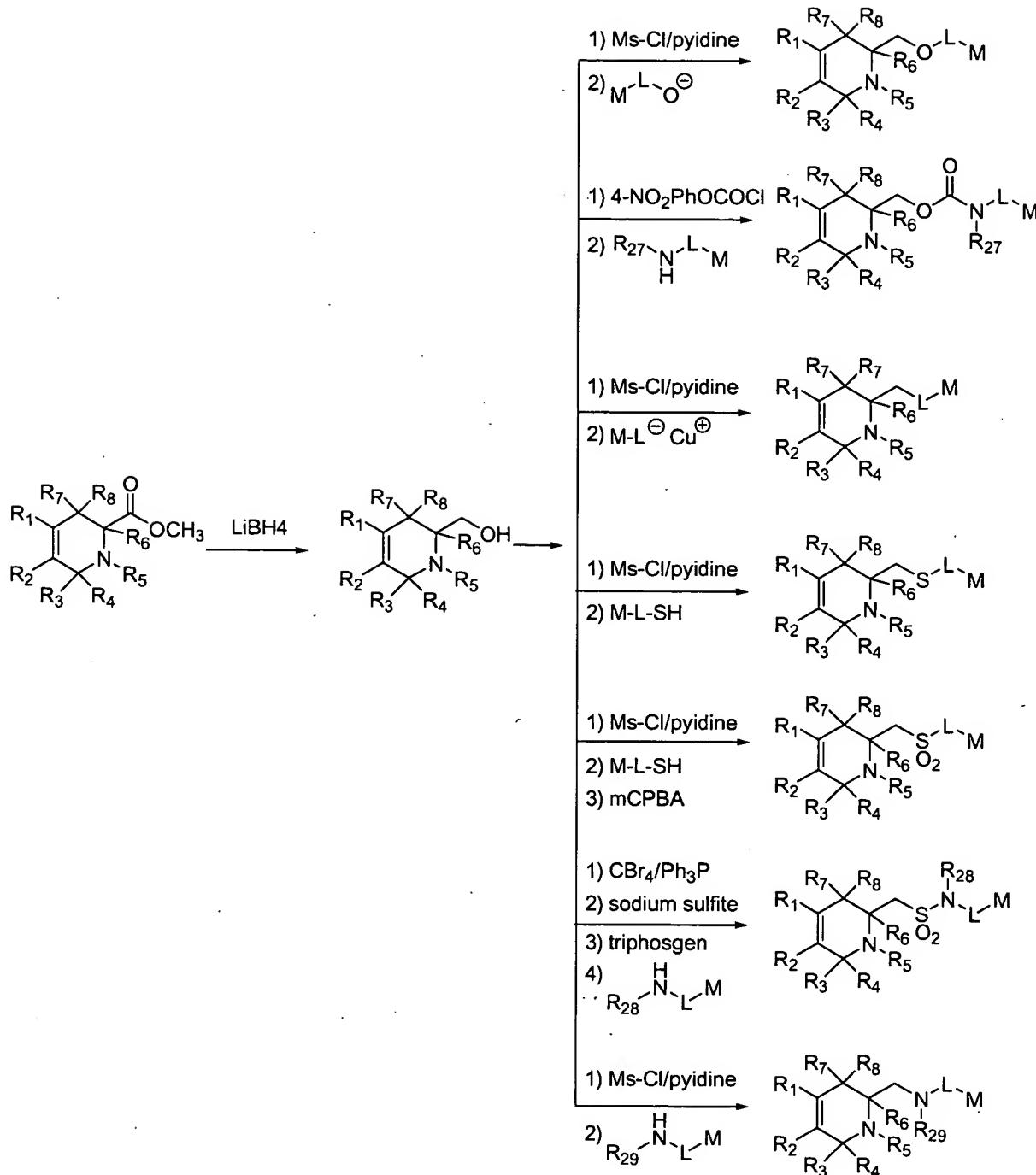
Scheme 2



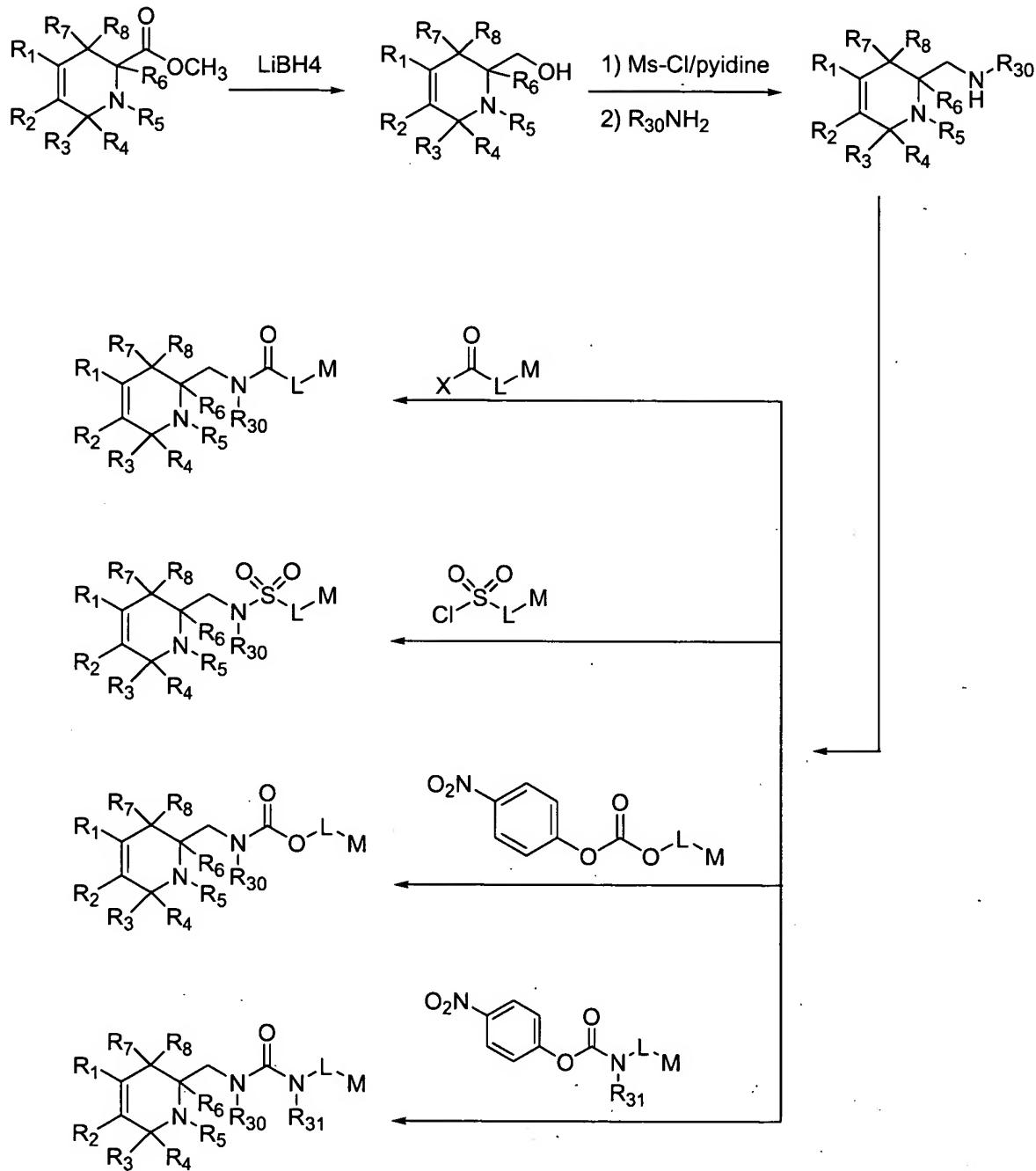
Scheme 3



Scheme 4



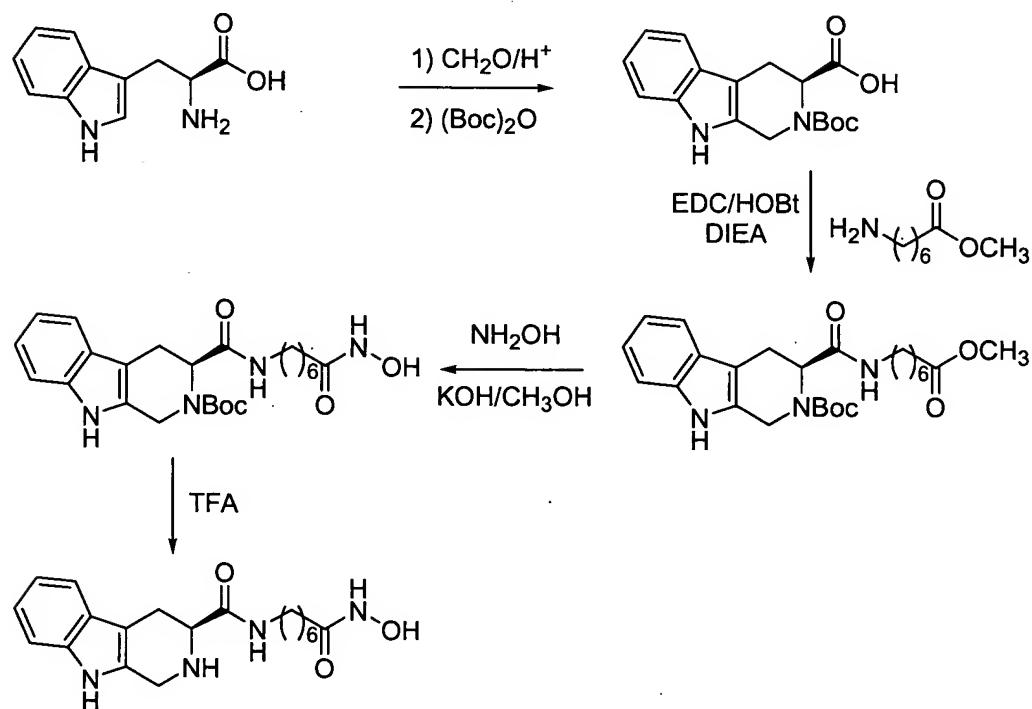
Scheme 5



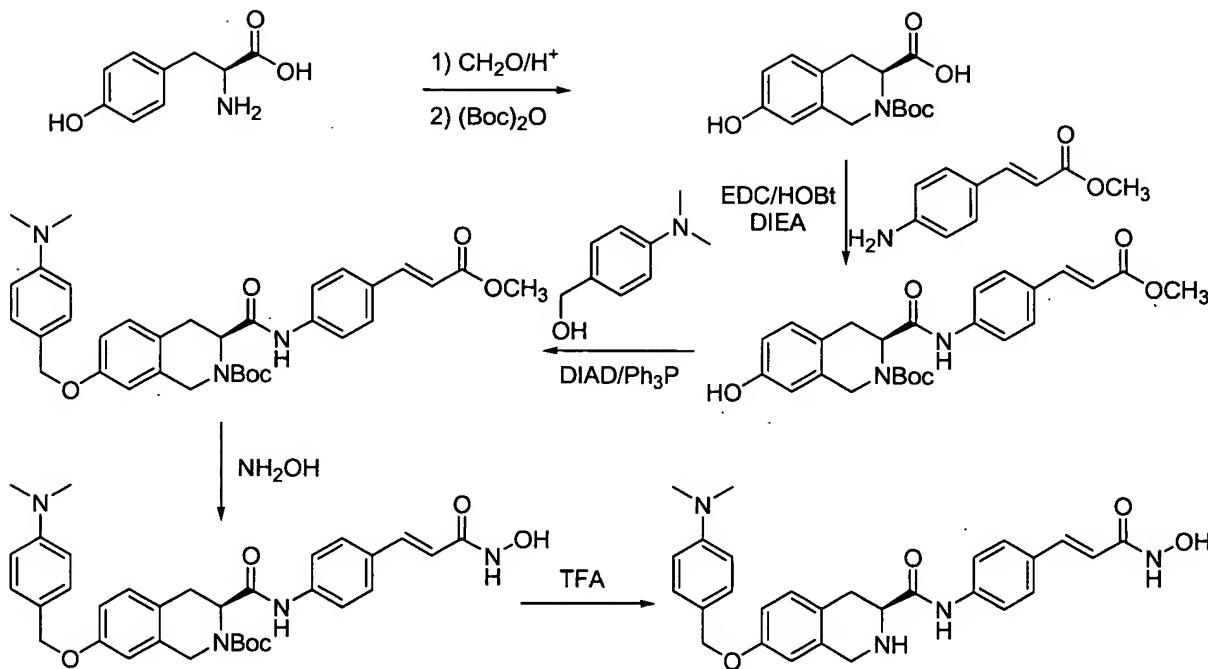
[00259] Reaction schemes 6 and 7 illustrate syntheses of particular HDAC inhibitors according to the present invention. These schemes are intended to be illustrative of synthetic

routes to these compounds, but it should be recognized that other routes may be used to arrive at compounds within the scope of the invention.

Scheme 6



Scheme 7



[00260] As can be seen from the above reaction schemes, a wide variety of different HDAC inhibitors can be synthesized by these reaction schemes. It is noted that the invention is not intended to be limited to the particular compounds provided in this example. Rather, a wide variety of other compounds according to the present invention having HDAC inhibitory activity may be synthesized by the reaction schemes provided as well as other reaction schemes that may be devised by one of ordinary skill in the art in view of the present teachings.

2. HDAC8 Activity Assay

[00261] Compounds according to the present invention may be screened for activity against one or more HDACs. Provided in this example is an assay for activity against HDAC8.

[00262] Purified HDAC8 may be obtained as follows. Residues 1-377 of SEQ. ID No. 1 which correspond to the entire sequence of human HDAC8 may be amplified by PCR and cloned into the *SmaI/HinDIII* site of pFastbac (Invitrogen) with a 6-histidine tag at the N-terminus. This DNA sequence is presented in Figure 3 as SEQ. ID No. 2. Expression in this vector generates a

fusion of HDAC8 residues 1-377 with an N-terminal 6x-histidine tag, the amino acid sequence of which is shown in Figure 4 as SEQ. ID. 3.

[00263] Recombinant baculovirus incorporating the HDAC8 construct may be generated by transposition using the Bac-to-Bac system (Invitrogen). High-titer viral stocks may be generated by infection of *Spodoptera frugiperda* Sf9 cells and the expression of recombinant protein was carried out by infection of *Trichoplusia ni* Hi5 cells (Invitrogen) in 10L Wave Bioreactors (Wave Biotech).

[00264] Recombinant protein may be isolated from cellular extracts by passage over ProBond (InVitrogen) resin. Partially purified extracts may then be further purified by high pressure liquid chromatography over a BioSep S3000 gel filtration resin. HDAC8 protein purity may be determined on denaturing SDS-PAGE gel. Purified HDAC8 may then be concentrated to a final concentration of 15 mg/ml and stored at 4°C in a buffer containing 25mM TRIS-HCl pH 7.6, 150mM NaCl, 1mM EDTA and 0.25 mM TCEP.

[00265] The inhibitory properties of compounds relative to HDAC8 may be determined using a white or black 384-well-plate format under the following reaction conditions: 25 mM Tris pH 8.0, 100 mM NaCl, 50 mM KCl, 0.1 mM EDTA, 0.01% Brij35, 0.1 mM TCEP. 50 uM tBoc-Lys(Ac)-AMC, 2% DMSO. Reaction product may be determined quantitatively by fluorescence intensity using a Fluorescence plate reader (Molecular Devices Gemini) with an excitation wavelength at 370 nm and emission at 480 nm (for white plates) or 465 nm (for black plates).

[00266] The assay reaction may be initiated as follows: 5 ul of 150 uM tBoc-Lys(Ac)AMC was added to each well of the plate, followed by the addition of 5 ul of inhibitor (2 fold serial dilutions for 11 data points for each inhibitor) containing 6% DMSO. 5 ul of HDAC8 solution may be added to initiate the reaction (final enzyme concentration was 10 nM for HDAC8). The reaction mixture may then be incubated at room temperature for 60 min, and quenched and developed by addition of 5 ul of 10 mM phenanthroline and 4 mg/ml trypsin (final concentration of phenanthroline is 2.5 mM, and trypsin is 1 mg/ml). Fluorescence intensities of the resulting reaction mixtures may be measured after a 30 minute incubation at room temperature.

[00267] IC50 values may be calculated by non-linear curve fitting of the compound concentrations and fluorescence intensities to the standard IC50 equation. As a reference point for this assay, suberanilohydroxamic acid (SAHA) showed an IC50 of 130 nM.

[00268] Figure 5 provides examples of HDAC inhibitors that were assayed according to the above assay and found to have better than 200 nM activity against HDAC8.

[00269] It will be apparent to those skilled in the art that various modifications and variations can be made to the compounds, compositions, kits, and methods of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.